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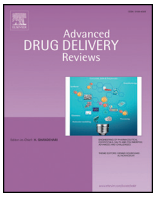

Thiolated polymers: Bioinspired polymers utilizing one of the most important bridging structures in nature

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ABSTRACT

Thiolated polymers designated “thiomers” are obtained by covalent attachment of thiol functionalities on the polymeric backbone of polymers. In 1998 these polymers were first described as mucoadhesive and *in situ* gelling compounds forming disulfide bonds with cysteine-rich substructures of mucus glycoproteins and crosslinking through inter- and intrachain disulfide bond formation. In the following, it was shown that thiomers are able to form disulfides with keratins and membrane-associated proteins exhibiting also cysteine-rich substructures. Furthermore, permeation enhancing, enzyme inhibiting and efflux pump inhibiting properties were demonstrated. Because of these capabilities thiomers are promising tools for drug delivery guaranteeing a strongly prolonged residence time as well as sustained release on mucosal membranes. Apart from that, thiomers are used as drugs per se. In particular, for treatment of dry eye syndrome various thiolated polymers are in development and a first product has already reached the market. Within this review an overview about the thiomer-technology and its potential for different applications is provided discussing especially the outcome of studies in non-rodent animal models and that of numerous clinical trials. Moreover, an overview on product developments is given.

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1. Introduction

Since their introduction in the life science arena in the late 1990s [1,2], thiolated polymers have attracted and inspired researchers from different disciplines by their versatile features resulting in some thousands of publications, numerous clinical trials and various marketed products. On the occasion of their 20th anniversary achievements so far are summarized and an outlook on future developments is taken.

Due to the covalent attachment of thiol moieties on polymers (Fig. 1) various properties are strongly improved [3]. Polymers without thiols are simply like proteins without cysteine. Soon after the first publications about thiolated polymers the term thiomers came additionally into use [4]. Their capability to form disulfide bonds – the likely most versatile bridging structure in nature – with endogenous proteins such as mucus glycoproteins, keratins or membrane-associated proteins as well as within their own polymer chains through a crosslinking process render them unique among advanced materials.

First thiomers were generated with the intention to improve the mucoadhesiveness of polymeric excipients for drug delivery. Well-established mucolytic drugs bearing a thiol group such as N-acetyl-cysteine break disulfide bonds within cysteine-rich substructures of mucus glycoproteins through thiol/disulfide exchange reactions. The mucolytic agent is thereby covalently attached to the mucus glycoprotein via a newly formed disulfide bond. Based on this observation it was assumed that not just small molecules but even polymers will be covalently attached to mucus glycoproteins following the same mechanism. Accordingly, cysteine was bound to polyacrylic acid via the same amide linkage as exhibited by N-acetylcysteine. Although this first synthesized thiomer showed just two to three fold improved mucoadhesive properties, a proof-of-concept was provided. In the same study the ability of thiomers to crosslink via the formation of inter- and intrachain disulfide bonds and to build up cohesive and robust three-dimensional networks due to a pH-dependent oxidation process was shown [2]. In a follow up study the technology was demonstrated to work also for other backbones such as polysaccharides [4]. Further properties of thiomers like permeation enhancing, enzyme inhibiting and efflux pump inhibiting properties were shown thereafter. In the 2010s especially the crosslinking of thiomers with dienes via thiol-ene reactions broadened their applicability [5,6]. Although having been developed for drug delivery systems, soon thiomers were investigated for other purposes like as drugs per se, as wound dressings, as scaffolds for tissue engineering and as injectable tissue-regenerative hydrogels. More recently, thiomers turned out to show also outside the life science arena great potential and meanwhile they are evaluated for various cosmetic and textile applications. Within this review an overview about the thiomer-technology and its versatile applications is provided.

2. Synthesis of thiolated polymers and chemistry behind

2.1. Rational design of thiolated polymers

Generally, the polymer backbone and the quantity as well as type of sulfhydryl groups have a substantial impact on the performance of thiomers.

In particular chain length, chain flexibility and charged substructures of the polymer backbone influence the ability to form disulfide bonds. The longer the chain length and the less flexible the polymer backbone is, the less reactive are thiomers [7]. As molecular movements of high molecular mass thiomers of low chain flexibility are comparatively slower, thiols need simply more time to get close to the target thiol or target disulfide to react. In addition, for such thiomers steric hindrances become more effective. If polymer chains repulse each other because of the same charge, the likelihood for thiols to get close to each other and to crosslink is also lowered [8,9]. Furthermore, polymer backbones can be either biodegradable such as polysaccharides and proteins or non-biodegradable such as poly(meth)acrylates or silicones. Biodegradable thiomers are from the toxicological point of view preferred over non-biodegradable thiomers. For the registration as drug, pharmaceutical excipient or medical device in case of biodegradable thiomers, however, toxicological studies of not just the thiomer itself but also of different degradation products have to be shown.

The reactivity of thiomers depends also on the degree of thiolation [10] and the type of sulfhydryl ligand [11]. As actually not the thiol but the thiolate anion is the reactive form, the pKa value of sulfhydryl ligands has a substantial impact on reactivity of thiomers. The lower the pKa value of thiol groups is, the higher is the concentration of thiolate anions at physiological pH. The rate of thiol/disulfide exchange reactions is inversely dependent on the pKa of the attacking sulfhydryl ligand, as the thiolate anion is a better nucleophile than the thiol [12]. Due to the linear-free energy relationship, however, nucleophilicity of the thiolate anion increases with its pKa causing an opposite effect on the overall reaction rate. In Fig. 2 the impact of both effects on the rate constant (K) is illustrated for the reaction of thiols of indicated pKa with oxidized glutathione. It demonstrates that decreasing the pKa of the attacking thiol results in an overall rate enhancement just until the thiolate anion becomes the dominant species ($pKa < 7$). Beyond this, the drop in nucleophilicity of the sulfur center determines the rate resulting in slower kinetics [13].

In Table 1 an overview about the pKa of different sulfhydryl ligands is provided. Thioamides that can convert to thiols such as 2-mercaptopyridine or 2-mercaptonicotinic acid are because of this tautomerism generally more reactive than simple alkyl thiols [11,14]. Using the Henderson-Hasselbalch equation the thiolate anion concentration at a certain pH can be readily calculated. As a physiological pH of 7.2 is likely of highest relevance, the ratio of thiolate anions to thiols of sulfhydryl

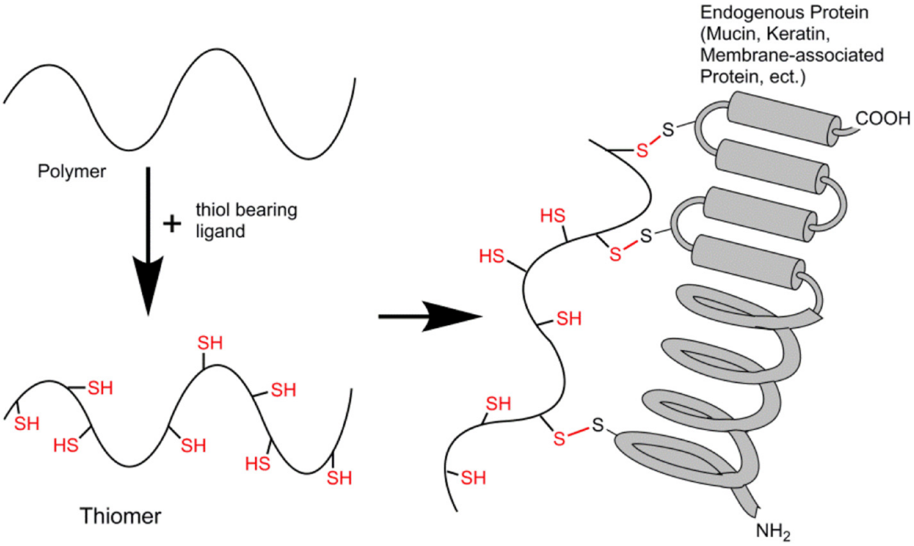


Fig. 1. Illustration of the transformation of a polymer to a thiomer and its adhesion to endogenous proteins via disulfide bond formations.

ligands is shown at this pH. The covalent attachment of sulfhydryl ligands as listed in Table 1, however, results in changes of their chemical structure due to conjugation and subsequently to altered pKa values. Furthermore, negatively charged neighboring groups on the thiomer such as carboxylic acid groups in case of polyacrylates tend to raise the pKa by destabilizing thiolate anions. In contrast, cationic neighboring groups such as amines in case of polyallylamine tend to lower the pKa by stabilizing thiolate anions. Hydrogen-bond donors of the local environment of the thiomer can also contribute to the stabilization of thiolate anions lowering the pKa. Via spectrophotometric titrations or via isothermal titration calorimetry the pKa of thiolated polymers can be accurately determined [15].

Apart from the sulfhydryl ligand itself also the neighboring groups to the thiol group or disulfide bonds have a tremendous impact on disulfide bond formation or thiol/disulfide exchange reactions. Anionic neighboring groups accelerate disulfide bond formation with thiols being flanked by cationic substructures and vice versa. On contrary, as illustrated in Fig. 3 thiol groups or disulfide bonds being neighbored by the same charged groups are hindered to form disulfide bonds or to participate in thiol/disulfide exchange reactions by electrostatic repulsion. The impact of the electrostatic environment on thiol/disulfide exchange reactions was for instance evaluated for cysteine being flanked by two neutral, by a neutral and a positive and two positive groups showing a rate constant for the reaction with the disulfide of the negatively charged Ellman's reagent of 367, 3,350 and 132,000 s^{-1} M^{-1}, respectively [16]. Based on these considerations the charge of groups neighboring thiols on target proteins are of course of high relevance. In case of mucus glycoproteins and keratins, for instance, a minor prevalence for negative charges can be found. Apart from the pKa value and the electrostatic environment various further parameters such as the redox potential of thiol groups have an effect on the reactivity of thiomers [17].

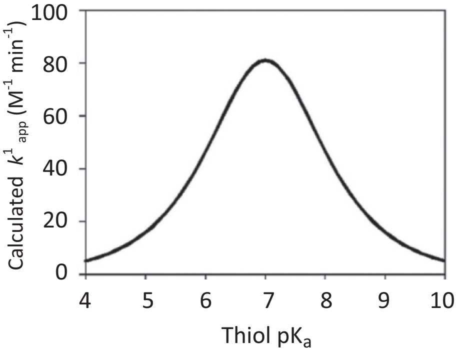
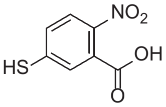
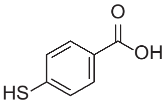
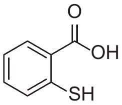
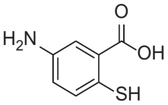
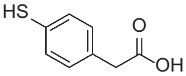
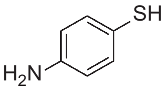
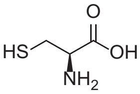
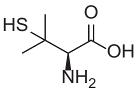
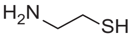
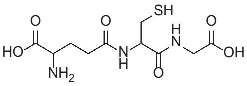
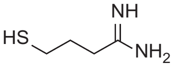
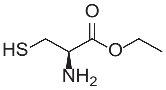
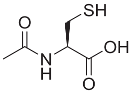
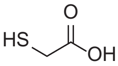
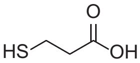
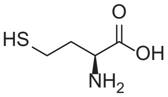


Fig. 2. Calculated rate constant (K) for thiol/disulfide exchange reactions as a function of thiol pKa at pH 7 based on the Brønsted relationship; adopted from Nagy [13].

According to this, reactivity of thiols on each polymer can be accurately adjusted. Generally high thiol reactivity does not necessarily go hand in hand with a high performance of the thiomer. In case of mucoadhesive properties a medium to low thiol reactivity seems even advantageous as the thiomer can to a high extent interpenetrate the mucus gel layer. Because of this more extensive interpenetration a much greater interface for disulfide bond formations between the thiomer and the mucus is generated. Because the mucus gel layer exhibits on most mucosal tissues such as the gastrointestinal, buccal and vaginal mucosa a pH gradient from 7 at the epithelium to <6 at the luminal surface [18], thiomers become more reactive the deeper they penetrate into the mucus gel layer. This effect contributes to high mucoadhesive properties and is certainly also advantageous when thiomers are supposed to interact with cell membrane-associated cysteine substructures. In contrast, highly reactive thiomers get already by the formation of disulfide bonds with mucins on the surface of the mucus layer immobilized strongly hindering the interpenetration process. Thiomers that are just anchored on the surface of the mucus layer and not in deeper mucus regions are furthermore much faster eliminated by the mucus turnover process [19]. For thiomers interacting with cell membrane-associated cysteine substructures high thiol reactivity is also disadvantageous as thiols are already inactivated on the way to the target cell by the formation of disulfides with other endogenous thiol substructures such as mucins or keratins. Even regarding the in-situ gelling features of thiolated polymers a too high reactivity of thiols is in most cases not beneficial, as a too rapid gelation of the polymer limits a proper distribution over the target tissue. On contrary, when the reactivity of thiols is too low they will not even at the right time and at the right place show sufficient potential as simply not enough new disulfide bonds are formed. The choice of the most suitable thiol reactivity is consequently always a compromise and depends on results of various key experiments such as mucoadhesion studies or permeation studies on freshly excised mucosa. The likely best compromise serving most purposes is a pKa around 8 as anyway also preferred by nature in form of cysteine.

Table 1
Reactivity of thiol ligands for thiol/disulfide exchange reactions and oxidation; pKa values of thiol groups were calculated using ADMET Predictor™ version 9.0.0.10.

Ligand	Structure	pKa	Ratio of thiolate anions to thiols at pH 7.2
5-Thio-2-nitrobenzoic acid		4.3	1 : 0.0013
4-Mercaptobenzoic acid		5.8	1 : 0.04
Thiosalicylic acid		6.0	1 : 0.06
5-Amino-2-mercaptobenzoic acid		6.4	1 : 0.16
4-Mercaptophenylacetic acid		6.6	1 : 0.25
4-Aminothiophenol		7.1	1 : 0.8
Cysteine*		***8.0***	***1 : 6.3***
Penicillamine		8.2	1 : 10
Cystamine		8.4	1 : 15.9
Glutathione		8.8	1 : 39.8
Thiobutylamidine		9.2	1 : 100
Cysteine ethylester		9.5	1 : 200
N-Acetylcysteine		9.7	1 : 316
Thioglycolic acid		10.0	1 : 631
3-Mercaptopropionic acid		10.2	1 : 1000
Homocysteine		10.5	1 : 1995

* Cysteine is the thiol structure used by nature and is bolded as kind of 'gold standard'.

Most thiolated polymers are susceptible to oxidation in aqueous media showing a pH that is not at least two levels below the thiol's pKa. Studies regarding pH-dependent disulfide formation of polyacrylate-cysteamine conjugates showed that the formation of disulfide bonds in acid media is widely quenched whereas increased disulfide bond formation occurred at a physiological pH of 7.4 [8]. Other thiolated polymers such as thiolated chitosans show similar sensitivity to a pH-dependent oxidation [20]. Due to the addition of antioxidants such as dithiothreitol, glutathione or cysteine this unintended oxidation of thiomers can be avoided. As these auxiliary agents, however, disturb disulfide bond formations between the thiolated polymer and proteins, the efficacy of thiomers is at least to some extent lowered [21,22]. Alternatively thiol groups can optionally be protected by the attachment of 2-mercaptopyridine and analogues. In particular mercaptonicotinic acid and mercaptonicotinamide are preferred over mercaptopyridine for safety reasons [23,24]. This S-protection does not just provide

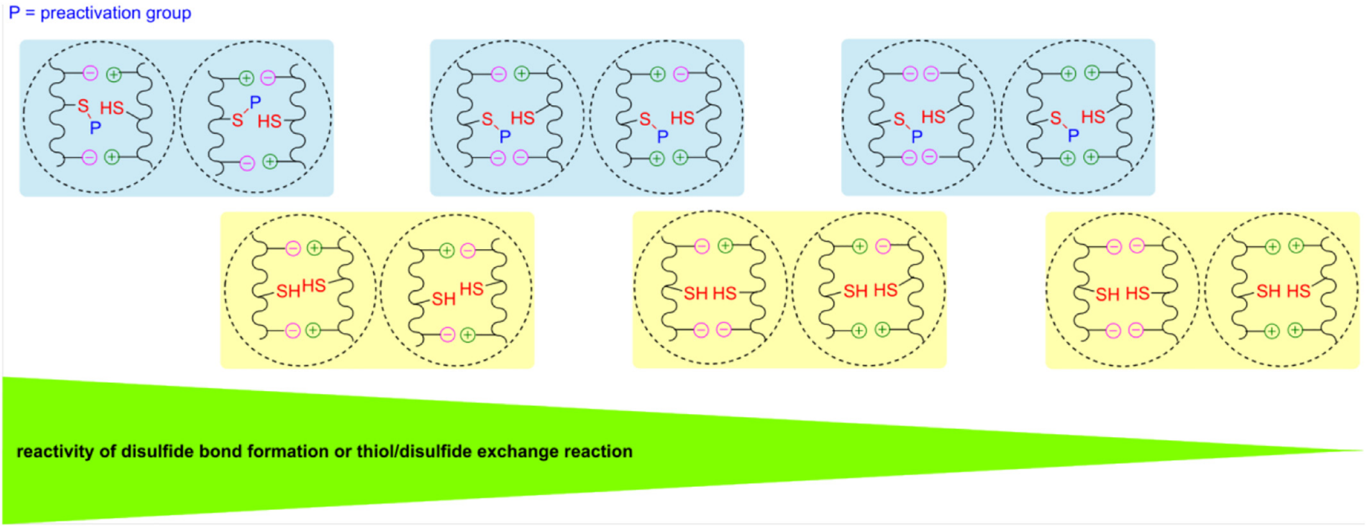


Fig. 3. Impact of neighboring groups on the reactivity of thiomers.

stability towards oxidation but increases also the reactivity of thiol groups. Because of the electron withdrawing effect of the π-system of the pyridine, the mercaptopyridine group is easily cleaved off liberating free thiol moieties with their complete initial activity. Menzel et al. established a version of a preactivated thiomer exhibiting a comparatively higher reactivity of the disulfide by the attachment of 2-mercaptonicotinic acid in its dimeric form to chitosan [11]. The additional nitrogen atom in conjugation over the aromatic rings evoked a 3.8-fold increase in reactiveness as ascertained in kinetic studies of disulfide exchange reaction in comparison to a conventional synthesized 100 % S-protected thiomer illustrated in Fig. 4. Besides, a 5.7-fold stronger rheological synergism with mucus than for the non-thiolated chitosan was verified. Free unprotected thiol groups react with disulfide bonds of proteins, whereas S-protected thiol groups are reacting with free thiol groups of proteins.

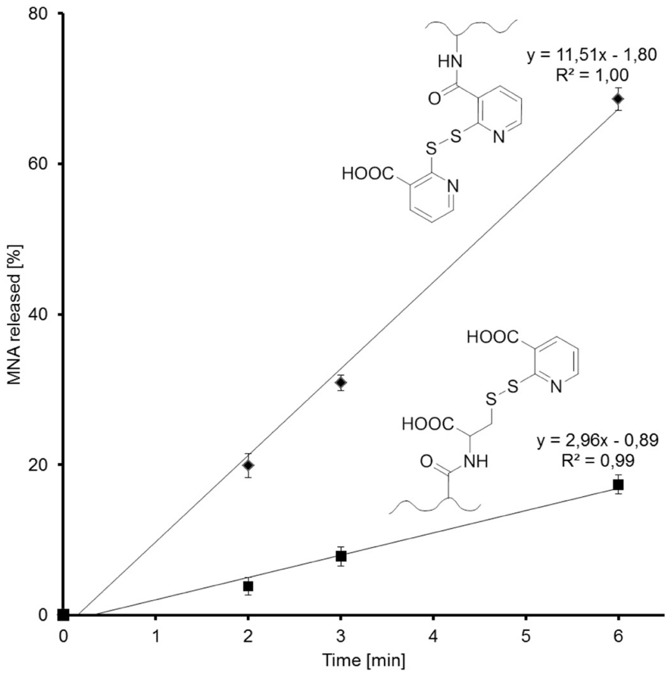


Fig. 4. Reactivity of two different S-protected thiomers. Because of the electron withdrawing effect of the pyridine substructure of mercaptonicotinic acid (MNA) those disulfide bonds being neighbored by two pyridine substructures show higher reactivity for thiol/disulfide exchange reactions with reduced glutathione at pH 6.8 than those being neighbored by just one; adopted from Menzel et al. [11].

As most target proteins such as mucus glycoproteins, keratins or membrane-associated proteins exhibit both disulfide and free thiol substructures, free as well as S-protected thiolated polymers are able to form disulfide bonds with these proteins [25]. In certain cases where primarily just disulfide bonds are available on target proteins free thiomers are more efficient and vice versa. As these *S-protected* thiomers are more reactive than unprotected thiomers, they are also referred as *preactivated* thiomers [24].

2.2. Synthesis of thiolated polymers

Generally, polymers can be thiolated by the immobilization of sulfhydryl ligands or the substitution of hydroxyl moieties on polymers by thiol groups. Apart from that thiomers can be generated by copolymerization reactions of various types of (thiolated) monomers. In Fig. 5 a synopsis of most relevant thiolation methods is provided.

2.2.1. Covalent attachment of sulfhydryl ligands

Generally commercially available preactivated reagents like γ-thiobutyrolactone or 2-iminothiolane are used to attach thiol substructures on amino bearing polymers. In contrast to 2-iminothiolane the formation of cyclic non-thiol products of the ligand during synthesis and storage can be avoided in case of isopropyl-S-acetylthioacetimidate [27]. Through reaction of imidates with primary amines amidine bonds are formed [41]. Because of the higher cationic character of amidines in comparison to amines, the cationic charge of such thiomers is consequently raised. This effect can be advantageous although a raised cationic character of polymers bears toxicological risks. Chitosan, for instance, precipitates in aqueous media above pH 6.5, whereas it remains stable even at pH 7.5 when bearing amidine substructures [20]. As free thiol groups can interfere with many coupling reactions they need to be protected. The most widely applied protective group is thioacetate such as in case of N-succinimidyl S-acetylthioacetate [28] that can be quantitatively eliminated with hydrolyzing agents such as NaOH, potassium carbonate or sodium methoxide after the coupling reaction. γ-Thiobutyrolactone is also useful for thiolation of polysaccharides as it forms esters with hydroxyl moieties of the polymer. Maleki et al., for instance, thiolated hemicellulose in this way [30].

Other coupling reactions are based on esterfication of sulfhydryl ligands bearing a carbonic acid substructure with hydroxyl groups of polymers mediated by hydrochloric acid [33,42,43]. The method is simple and no byproducts are formed. Alternatively, the carboxylic

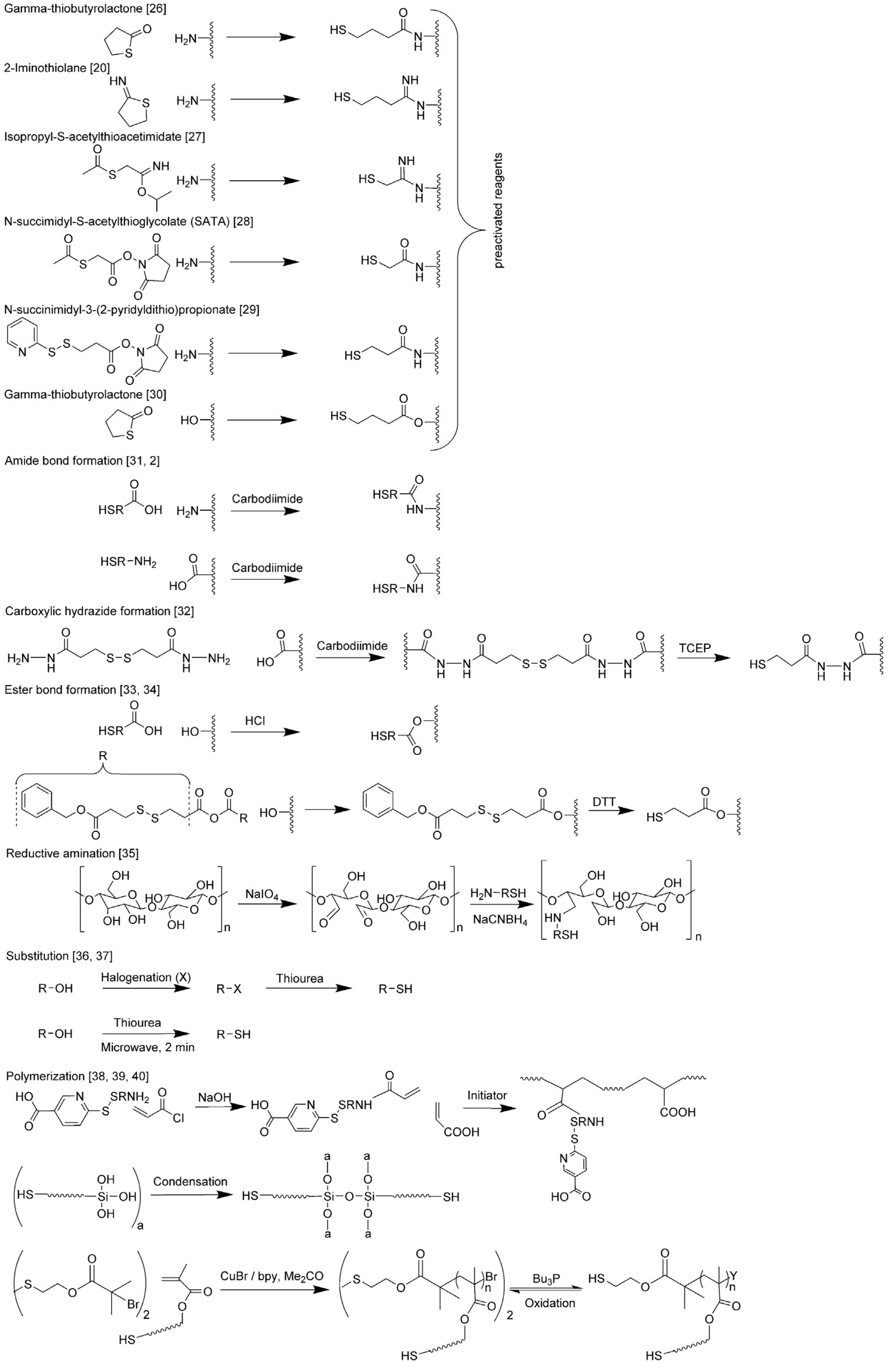


Fig. 5. Methods for the synthesis of thiolated polymers Preactivated reagents [26,20,27–30] amide bond [31,2] carboxylic hydrazide [32] ester bonds [33,34] reductive amination [35] substitution [36,37] polymerization [38–40].

acids can be activated by the formation of anhydrides to facilitate esterfication [34].

Alternatively, sulfhydryl ligands are often attached to polymers via carbodiimide mediated amide or ester bond formations. Such reactions can be performed both in aqueous media and in organic solvents. Zhu et al. could prevent O-acylisourea ester hydrolyzation in water by performing the reaction in N,N-dimethylformamide obtaining a 6-fold higher degree of thiolation [44]. Employing anhydrous synthesis conditions with a solvent mixture of N,N-dimethylformamide/dichloromethane and applying N,N′-diisopropylcarbodiimide increased coupling rates of cysteine to poly(acrylic acid) by 4-fold compared to the aqueous control synthesis [45]. In order to improve the reaction efficacy active esters such as (sulfo)-N-hydroxysuccinimide esters or N-hydroxybenzotriazole (HOBt) esters can be formed as intermediate products [46,47]. As HOBt derivatives have generally been regarded as potential explosives, they might be replaced in the future by ethyl(hydroxyimino)cyanoacetate or COMU ((1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate). Thiol groups are also often S-protected by acetylation for these reactions. Alternatively ligands are used as disulfide dimers being reduced after the coupling reaction [34,48]. First S-protected thiomers having been synthesized as described above were obtained by the reaction of thiomers with dimers of 2-mercaptonicotinic acid or 2-mercaptonicotinamide. As via this method an entire S-protection cannot be achieved, an alternative method was developed by Hintzen et al.. By the immobilization of already S-protected sulfhydryl ligands to the polymer backbone via a carbodiimide mediated reaction remaining free mercapto groups can be excluded [49].

In case of oligo- and polysaccharides aldehyde substructures can be formed through an oxidative ring opening of monosaccharides and reductive amination with thiol bearing amines such as cysteamine [50,35]. This method, however, leads to the formation of secondary amino substructures providing a cationic character of the polymer that can from the toxicological point of view be disadvantageous as most cationic polymers like polyallylamine, polylysine or polyarginine exhibit pronounced cytotoxicity. Furthermore, the cationic character of the polymer can cause strong interactions with anionic drugs and a restricted cross-linking because of ionic repulsions. Additionally, integrity of ring structures within the polysaccharide backbone cannot be maintained. In case of cyclodextrins this ring opening seems to be even advantageous as drug solubilizing properties of these oligosaccharides can be improved [51].

2.2.2. Substitution of hydroxyl groups by thiol groups

As many polymers exhibit hydroxyl groups such as polysaccharides, these alcohols can be simply substituted by thiol groups. In a first step hydroxyl groups are substituted by bromine or iodine, that is in the following step replaced by a thiol group via reaction with thiourea [36]. Hydroxyethylcellulose, for example, was brominated with N-bromosuccinimide-triphenylphosphine using lithium bromide in N,N-dimethylacetamide. In the following bromo substructures were replaced by thiol groups through reaction with thiourea [52]. The same synthetic pathway can also be applied for cyclodextrins [36]. Apart from oligo- and polysaccharides poly(vinyl alcohol) was thiolated by the same method [53]. More recently, chitosan was directly thiolated with thiourea just under microwave irradiation [37]. As a halogenation is not necessary and the method takes just a few minutes, it seems advantageous.

2.2.3. Co-polymerization reactions

In contrast to the attachment of sulfhydryl ligands and substitution reactions, the variety and flexibility to design thiomers of choice is much greater in case of co-polymerization reactions. Once an appropriate thiolated monomer has been generated, it can be co-polymerized with different types of other monomers in various ratios. As most polymerization reactions are free radical mediated and free thiol groups are radical scavengers, however, thiol groups need to be S-protected. In case of alkene polymerizations this S-protection is also necessary to avoid thiol-ene reactions as described in detail in the following chapter. In Table 2 S-protected thiol bearing alkene monomers are summarized. Solhi et al. created for instance S-protected thiomers based on polymerization of monomers with vinyl substructures. First, a mixed disulfide of 6-mercaptonicotinic acid (6-MNA) and cysteamine was reacted with acryloyl chloride leading to 6-(2-acryloylamino-ethyldisulfanyl)-nicotinic acid (ACENA) that was in the following co-polymerized with acrylic acid [38]. ACENA was recently also co-polymerized with N-vinylpyrrolidone to obtain S-protected polyvinylpyrrolidone [54]. Alternatively, disulfide dimers can be co-polymerized with other monomers and thereafter reduced. Acrylamide, N-phenylacrylamide and bisacryloylcystamine, for instance, were co-polymerized and thereafter reduced with dithiothreitol (DTT) [55]. Besides dithiothreitol sodium borohydride and tris(2-carboxyethyl)phosphine (TCEP) turned out as useful reducing agents [56,57].

In case of condensation reactions like with (3-mercaptopropyl) trimethoxysilane S-protection is not necessary [73–75]. An example for a thiomer having been gained via condensation reaction is dimethicone/mercapto propyl methicone copolymer as illustrated in Fig. 6 [76,77]. Condensation reactions offer the additional possibility to directly form thiolated nanoparticles. Recently, Al Mahrooqi et al. could demonstrate that even the size of thiolated nanoparticles can be accurately controlled by such reactions [39]. Despite the great flexibility of this approach polymerization and condensation reactions are nevertheless rarely used by pharmaceutical industry as standardization and upscale is much more complex and time consuming than the modification of already well-characterized polymers. For other industries such as the cosmetic industry, however, this does not seem to be an issue [77].

Generally, storage stability of thiomers is provided in solid state [78]. In case of semisolid or liquid formulations, however, thiomers are oxidized over time [8]. In order to overcome this stability problem they can be either S-protected as described above or they can be stored under inert conditions in the same way as drugs that are subject to oxidation as well.

3. Reactions of thiolated polymers

Thiol groups can undergo either nucleophilic (thiol-disulfide exchange, Michael addition) or radical (oxidation, thiol-ene) reactions. As both reactions are promoted by thiolate anions, efficiency and kinetic are highly pH dependent. An overview about these reactions is provided in Fig. 7.

In case of thiol-disulfide exchange reactions disulfide bonds are the target of nucleophilic attack by thiolate anions. If symmetric disulfides are attacked, the new disulfide is formed with both sulfur atoms without any preference. In case of asymmetric disulfides, however, the formation of more stable new disulfides is favored. The more electron-donating groups are surrounding the disulfide bond, the more stable it is. For the application of most thiomers a rapid formation of highly stable disulfides is advantageous. As on the one hand a rapid formation of disulfides is favored by thiols with electron-withdrawing neighboring groups and the stability of the newly formed disulfide is on the other hand improved by electron-donating groups, the design of thiomers ends up with a compromise. In case of mucoadhesion, for instance, a too fast formation of new disulfide bonds seems even disadvantageous as the interpenetration of the thiomer into the mucus gel layer additionally improving adhesion through polymer chain entanglements is hindered by already formed new disulfide bonds. In other cases the residence time of thiomers with low reactivity is simply too short to form new disulfides at the target site. In contrast to the influence of electron-donating and –withdrawing groups, oppositely charged groups on the attacking thiol and on the disulfide as illustrated in Fig. 3 are contributing to both reactivity and stability in the same manner.

Table 2
Thiolated mono-, di- and oligomers used for synthesis of thiomers.

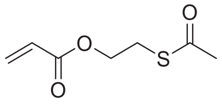
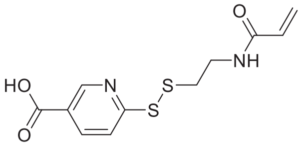
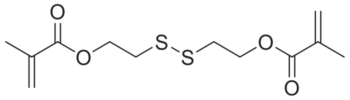
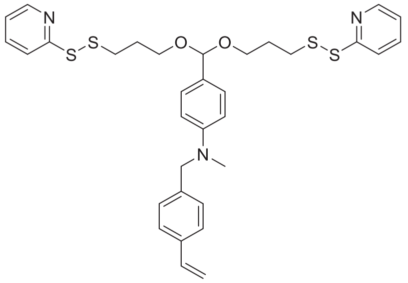
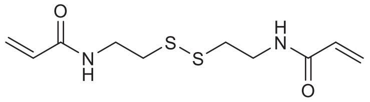
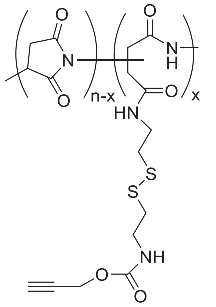
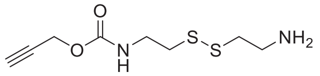
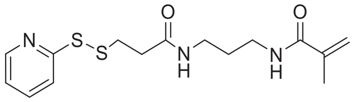
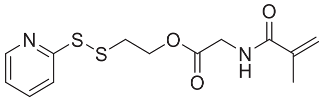
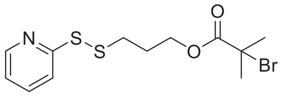
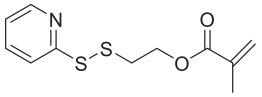
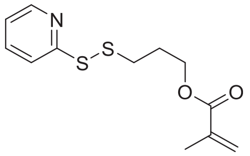
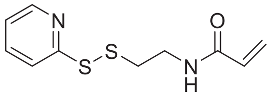
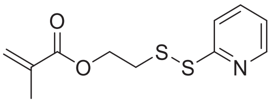
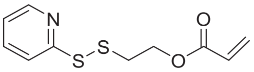
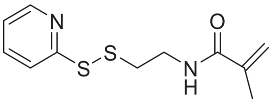
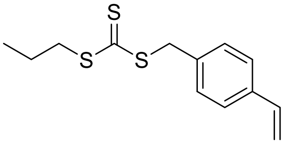
Mono-, di- and oligomers	Chemical structure	References
2-(Acetylthio)ethylmethacrylate		[58,59]
6-(2-Acryloylamino-ethyldisulfanyl)-nicotinic acid		[38]
Bis(2-methacryloyloxyethyl)disulfide		[60]
4-(Bis(3-(pyridin-2-yldisulfaneyl)propoxy)methyl)-N-methyl-N-(4-vinylbenzyl)aniline		[61]
N,N′-Cystaminebisacrylamide		[62–64]
[(Propargyl carbamate)-cystamine]-α,β-aspartamide (Asp-SS-AL)		[65]
Prop-2-yn-1-yl (2-((2-aminoethyl)disulfaneyl)ethyl)carbamate		[65]
N-(3-(3-(Pyridin-2-yldisulfanyl)propanamido)propyl)methacrylamide		[28]
2-(2-Pyridin-2-yldisulfanyl)ethyl-2-(methacrylamido)acetate		[28]
3-(Pyridin-2-yldisulfaneyl)propyl 2-bromo-2-methylpropanoate		[66]
2-(Pyridin-2-yldisulfaneyl)ethyl methacrylate		[67–69]
3-(Pyridin-2-yldisulfaneyl)propyl methacrylate		[70]
Pyridyldisulfide ethylacrylamide		[70]

Table 2 (*continued*)

Mono-, di- and oligomers	Chemical structure	References
Pyridyldisulfide ethylmethacrylate		[67–69]
2-(Pyridin-2-yldisulfaneyl)ethyl acrylate		[71]
N-(2-(Pyridin-2-yldisulfaneyl)ethyl)methacrylamide		[71]
S-(4-Vinyl) benzyl S′-propyltrithiocarbonate		[72]

Oxidation reactions of thiomers depend on the concentration of thiolate anions and their accessibility to each other on the polymer. Thiols being neighbored by groups of the same charge repulse each other and are less oxidized than those being neighbored by oppositely charged groups. Furthermore, the oxidation of thiomers can be accelerated by sonication [79], enzymes [80] and oxidants [81].

Crosslinking due to the addition of thiol groups to alkenes either by a nucleophilic addition (Michael addition) or a radical-mediated process is becoming increasingly important within the development of novel biomaterials such as particles and hydrogels for drug delivery, dental restorative resins, biomimetic hydrogels and hydrogels for tissue engineering and regeneration [82–85]. Although thiol-ene reactions are known since hundred years and are used for polymerizations since several decades [82], they came just a few years ago into use for the crosslinking of thiomers [5,6]. Most thiol-ene reactions are conducted under radical conditions being photochemically initiated. Reactions are very rapid and can be complete even within a few seconds. In Table 3 alkenes useful for the crosslinking of thiomers are summarized although the reaction can be performed with virtually any olefin bond. Terminal enes show generally higher reactivity towards hydrothiolation than internal enes. On this way, unique crosslinking structures with improved mechanical properties can be easily generated by an environmentally harmless fast and selective reaction. The Michael addition involves the nucleophilic addition of a thiol to a α,β-unsaturated carbonyl compound such as acrylate or methacrylate groups [86–88]. *In situ* chemical crosslinking is selective under physiological conditions without the need of toxic adjuvants [89]. Hydrogels are for example formed by the reaction between thiol-modified sodium hyaluronate and thiolated gelatin with diacrylated PEG (PEGDA) [90]. The light initiated thiol-ene chemistry starts with the thiyl radical upon abstraction of a hydrogen atom followed by its addition to the alkene and by proton abstraction from a thiol-containing molecule to generate a new thiyl radical. This chain transfer process is highly efficient whereby a single radical can cause up to tens of thousands additions [91,92].

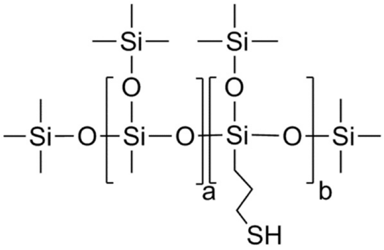


Fig. 6. Dimethicone/mercapto propyl methicone copolymer; adopted from Hawkins et al. [77].

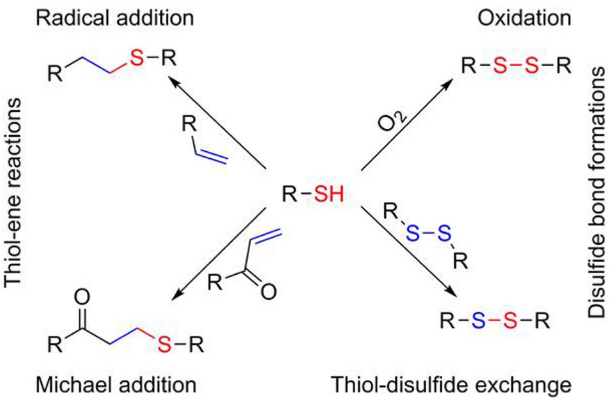


Fig. 7. Reactions of thiolated polymers.

4. Key features of thiolated polymers

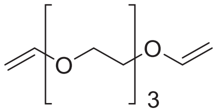
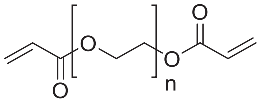
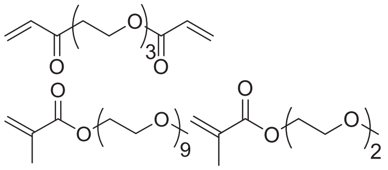
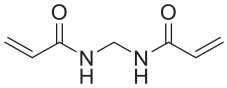
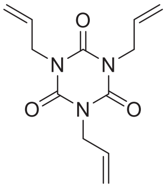
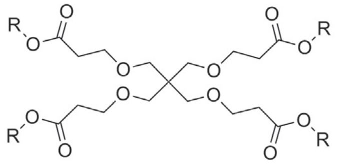
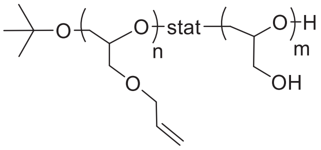
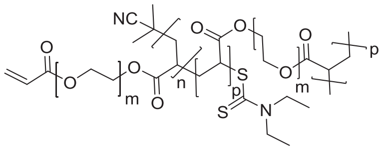
Key features of thiomers are primarily based on their capability to form disulfide bonds with endogenous proteins and their *in situ* crosslinking properties. An overview about the versatile applicability of thiolated polymers is provided in Fig. 8.

4.1. Bioadhesion

4.1.1. Mucoadhesion

Mucosal surfaces are easily accessible and therefore essential application sites for local therapy as well as non-invasive systemic drug delivery. Mucoadhesion is generally provided by the interaction of polymers with mucins via either weak non-covalent bond formations such as hydrogen bonds, van der Waal's forces, ionic and hydrophobic interactions or interpenetration processes [99]. Because of their capability to build disulfide bonds with cysteine substructures of the mucus gel layer, thiomers as well as their S-protected analogues provide stronger adhesive linkages by thiol/disulfide exchange reactions or oxidation of thiols. Evidence for these disulfide bond formations was first provided by Leitner et al. [21]. Furthermore, disulfide bond formation can occur between thiomer chains in a time dependent and controllable manner. Due to this stable crosslinking, a high cohesive polymer network is provided going hand in hand with minor adhesive bond failures. In addition, adhesive properties are enhanced by interpenetration. As soon as

Table 3
Overview of alkenes used for the crosslinking of thiomers.

Crosslinker	Abbreviation	Structure	References
Tri(ethylene glycol) divinyl ether	TEGDVE		[93,94]
Poly(ethylene glycol)diacrylate	PEGDA		[6,95]
Poly(ethylene glycol) based hyperbranched copolymer of high content of acrylate vinyl moieties	PEGMEMA 475 –MEO 2 MA–PEGDA 258		[5]
N,N′-Methylenebis(acrylamide)	MBAA		[30]
Triallyl-1,3,5-triazine-2,4,6-trione	TTT		[93]
Tetra PEG-tetra-acrylates	TetraPAc8	 R=$(CH_2CH_2O)_nCH_2CH_2OC(O)C(CH_3)C{=}CH_2$	[96]
Allyl functionalized poly(glycidol)	P(AGE-co-G)		[97]
Multi-acrylate hyperbranched PEG			[98]

the polymer penetrates the mucus gel layer, stabilizing disulfide bonds are built and the thiomer is effectively anchored within the mucus mesh work as illustrated in Fig. 9.

The impact of thiolation on the performance of polymers compared to the unmodified controls has been investigated in numerous studies by research groups worldwide. Even more than 100-fold improved mucoadhesiveness due to thiolation is reported [100]. Eye drops containing ^{124}I-labeled chitosan–N-acetylcysteine (chitosan–NAC) were examined in rabbits enabling microPET technology. Expansion in ocular residence time to almost 24 h and improved biodistribution were assigned for eye drops containing thiolated chitosan whereas an aqueous solution of Na^{124}I was cleared from the mucosa within an hour [101]. This strongly prolonged ocular residence time of chitosan-NAC was in the following confirmed in clinical trials [102]. Generally, S-protected thiomers show higher mucoadhesive properties than unprotected thiomers. Iqbal et al. evaluated in this connection the impact of S-protection on thiolated poly(acrylic acid) showing that the improved mucoadhesive features obtained by thiolation can be even further improved via S-protection [24]. Various review articles report about the superior mucoadhesive properties of thiolated polymers and S-protected thiomers e.g. [103] [104–107]. An overview about so far developed mucoadhesive thiolated polymers is provided in Table 4.

More recently, decoration of nanosized carriers such as nanoparticles, liposomes, self-emulsifying drug delivery systems (SEDDS) and micelles with thiol functionalities moved in the focus of research. By forming or coating nanocarriers with thiolated polymers mucosal residence time can be strongly prolonged and drug uptake consequently increased. Fabiano et al., for instance, showed a 2-fold higher oral bioavailability of a macromolecular model drug with thiolated nanoparticles vs. non-thiolated nanoparticles because of their improved mucoadhesive properies [125]. Barthelmes et al. showed in another *in vivo* study 4-fold higher mucoadhesive features of thiolated nanocarriers in comparison to non-thiolated nanocarriers on intravesical mucosa of rats [239]. Similar results regarding the mucoadhesive features of thiolated nanocarriers on intravesical mucosa were obtained by Cook et al. [59]. Liposomes can be coated with anionic and cationic thiolated polymers in order to improve their efficacy as

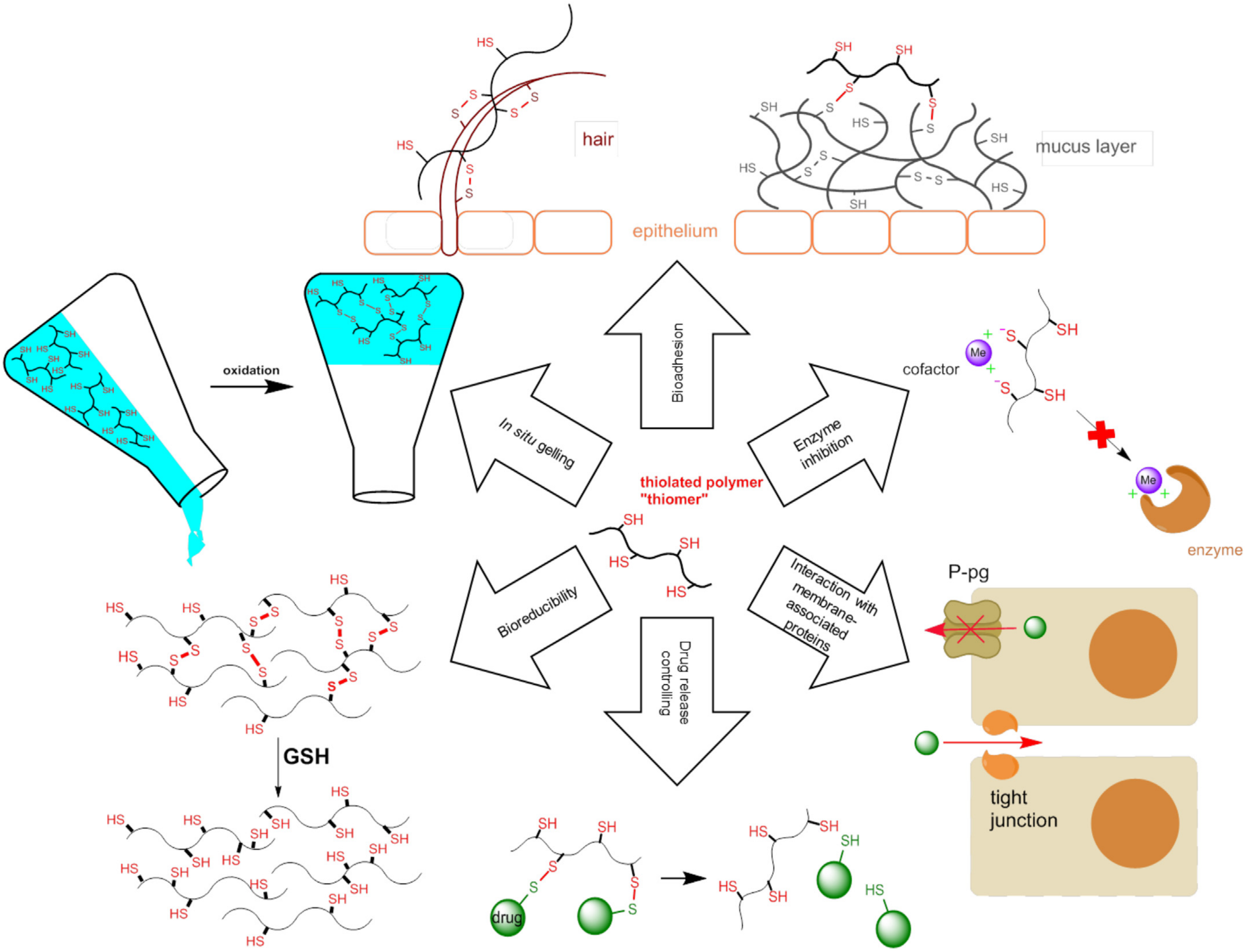


Fig. 8. Overview about the versatile applicability of thiolated polymers.

drug delivery systems e.g. [240–242]. In case of SEDDS, thiomers are incorporated in the lipophilic pre-concentrate assembling on the surface of the oily droplets formed during the dilution process in aqueous media [192]. Upon oral administration of thiolated chitosan SEDDS containing insulin, for instance, serum insulin was significantly increased compared to an orally dosed insulin solution [243]. Furthermore, improved mucoadhesive properties were shown for thiolated polymeric micelles [244].

Other more recent developments are focusing on the improvement of mucoadhesive properties of cyclodextrins via thiolation. Ijaz et al. obtained a mucoadhesive β-cyclodextrin (β-CD-Cys) conducting a two-step synthesis with oxidative ring opening prior to covalent linkage

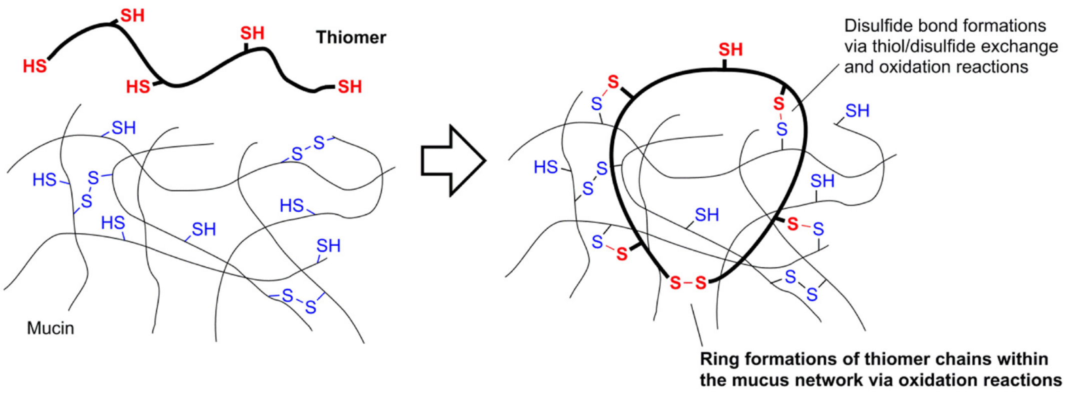


Fig. 9. Illustration of mechanisms being responsible for high mucoadhesive properties of thiolated polymers.

Table 4

Overview about different types of thiomers; MA = mucoadhesive; XL = crosslinking; PE = permeation enhancing; EPI = efflux pump inhibiting.

Type of polymer backbone	Polymer backbone	Type of thiolation	Preactivation	Properties	Applications	Products	Representative references (limited to 3 references; for further references please contact the corresponding author)
Polysaccharides	Hyaluronic acid	Attached ligands: cysteine; 3-mercapto propionic acid; 3-mercapto propanoic dihydrazide; 4-mercapto-butyric dihydrazide	Preactivated with 2-mercaptonicotinic acid	MA; XL; PE	Drug delivery; tissue engineering; regenerative medicine	Blafar Fast-Gelling Thiolated Hyaluronic Acid	[108–110]
	Carboxymethyl hyaluronic acid	Attached ligands: 3-mercapto-propanoic dihydrazide		MA, XL	Tissue engineering; wound healing	Hystem® Hystem® -HP Glycosil® Heprasil® Renevia® Premvia™ ReGlyde™	[111–113]
	Chitosan	Attached ligands: thiolglycolic acid; N-acetyl cysteine; glutathione; 4-thiobutylamidine; 3-mercaptopropionic acid; 6-mercaptonicotinic acid; 11-mercaptoundecanoic acid	Preactivated with 6-mercaptonicotinamide	MA; XL; PE; EPI	Drug delivery; regenerative medicine; tissue engineering	Lacrimera®	[114–116]
	Glycol chitosan	Attached ligands: N-acetylcysteine; glutathione; thioglycolic acid; 3-mercaptopropionic acid, 3,3'-dithiodipropionic acid	Preactivated with 2-mercaptonicotinic acid	MA, PE	Drug delivery		[117,118,119]
	Trimethyl-chitosan	Attached ligand: cysteine; homocysteine		MA; XL; PE	Drug delivery		[120–122]
	Triethyl chitosan	Attached ligand: cysteine		MA; PE	Drug delivery		[123]
	N,O-[N,N-diethylaminomethyl (diethyl-dimethylene-ammonium) methyl] chitosans	Attached ligands: thioglycolic acid; 3-mercapto propionic acid; 3-(2-pyridyldithio)propionic acid	Preactivated with 6-mercaptonicotinamide; pyridine-2-thiol	MA; PE	Drug delivery		[124–126]
	Alginate	Attached ligands: cysteine; thioglycolic acid		EPI; MA; XL	Drug delivery; wound healing		[127–130]
	Pectin	Attached ligands: thioglycolic acid; cysteine; 4-aminothiophenol		MA; XL; EPI	Drug delivery		[131–133]
	Heparin	Attached ligands: cysteamine; 4-thiobutylamidine; 3-mercapto propanoic hydrazide		XL	Tissue engineering, regenerative medicine	Heprasil®	[134–136]
	Chondroitin sulfate	Attached ligands: 3-mercapto propanoic hydrazide		XL	Regenerative medicine		[137,57,138]
	Gellan gum	Attached ligands: thioglycolic acid; cysteine; 4-thiobutylamidine		MA;XL	Drug delivery; tissue engineering		[139–141]
	Carboxymethyl gellan gum	Attached ligands: thioglycolic acid		MA; XL	Drug delivery		[142]
	Carrageenan	Substitution of hydroxyl groups with thiol groups		MA; EPI,	Drug delivery		[143]
	Starch	Ring opening and reductive amination; Attached ligands: thioglycolic acid; Substitution of hydroxyl groups with thiol groups		MA	Drug delivery		[144–146]
	Pullulan	Attached ligands: cysteamine; mercaptosuccinic acid	Preactivated with 6-mercaptonicotinamide	MA	Drug delivery		[147–149]
	Glycogen	Attached ligand: cysteine	Preactivated with 2-mercaptonicotinic acid	MA	Drug delivery		[150–152]
	Xyloglucan	Attached ligands: thioglycolic acid		PE; MA; XL	Drug delivery		[153–155]
	Cellulose	Substitution of hydroxyl groups with thiol groups; Attached ligands: thioglycolic acid; 6-amino-1-hexanethiol		XL	Functionalized cellulose films		[156–159]
	Carboxymethyl cellulose	Attached ligands: cysteine,	Preactivated with 2,2'-dithiodinicotinic acid	MA; XL; PE; EPI	Drug delivery		[160–162]
	Hydroxy ethylcellulose	Attached ligands: cysteamine; Substitution of hydroxyl groups with thiol groups	Preactivated with 6-mercaptonicotinamide	MA; PE; XL	Drug delivery		[35,52,163]
	Hydroxy propylcellulose	Attached ligands: cysteamine		MA; XL	Drug delivery		[164]

	Dextran	Ring opening and reductive amination with cysteamine; Attached ligands: mercaptopropionic acid			Drug delivery		[50,165]
	Carboxymethyl dextran	Attached ligands: cysteine, cysteamine			Drug delivery		[166–168]
	O-Acetyl-galactoglucomannan	Attached ligand: 4-mercapto butyric acid		XL			[30]
	Flaxseed mucilage	Attached ligand: thioglycolic acid		MA	Drug delivery		[169]
	Arabinoxylan	Attached ligands: thioglycolic acid		MA; PE	Drug delivery		[170]
	Xanthan gum	Attached ligands: thioglycolic acid; 3-mercapto propionic acid; cysteine	Preactivated with 2-mercaptonicotinic acid	MA; XL			[171–173]
	Fucoidan	Attached ligands: cysteamine		PE; EPI	Drug delivery		[174]
Cyclodextrins	alpha-Cyclodextrin	Attached ligands: cysteine	Preactivated with 2-mercaptonicotinic acid	MA	Drug delivery		[51,175]
	beta-Cyclodextrin	Attached ligands: cysteine; cysteamine; mercaptohexamethylene; Substitution of hydroxyl groups with thiol groups		MA	Drug delivery, food additive/antioxidant; biomedicine		[176–179]
	gamma-Cyclodextrin	Substitution of hydroxyl groups with thiol groups	Preactivated with 2-mercaptonicotinic acid	MA	Drug delivery		[180,181]
Poly(meth) acrylates	Carbomer	Attached ligands: cysteine,	Preactivated with 2-mercaptonicotinic acid	MA; PE XL; EPI	Drug delivery; diagnostics		[182–184]
	Polycarbophil	Attached ligands: cysteine; N-acetylcysteine		MA; XL; PE	Drug delivery		[4,185,186]
	C10-30 alkyl-modified carbomer	Attached ligands: cysteine; homocysteine; 4-aminothiophenol	Preactivated with 2-mercaptonicotinic acid	MA	Drug delivery		[187,188]
	Eudragit L 100	Attached ligands: cysteine	Preactivated with 2-mercaptonicotinic acid; 6-mercaptonicotinamide		Drug delivery		[189–191]
	Eudragit S 100	Attached ligands: cysteamine					[192]
	2-thioethylacrylate/ 2-hydroxyethylmethacrylate co-polymers	2-mercaptoethyl		MA	Drug delivery		[58,59]
	Poly(methacrylic acid-co-sodium acrylamidomethyl propane sulfonate	Attached ligands: cysteine	Preactivated with 2-mercaptonicotinic acid	MA; XL	Drug delivery		[193]
Poly (acrylamide) copolymers	Poly(acrylamide) copolymer	Attached ligands: cysteamine; *N*,*N*-bis (acryloyl)-cystamine as linker			Vitreous replacement		[194,55,195]
Silicones	Amino-modified silicone oil	Attached ligands: cysteine; thioglycolic acid; 3-mercaptopropionic acid; 11-mercaptoundecanoic acid; 1,6-hexanedithiol	Preactivated with 2-mercaptonicotinic acid				[196–198]
	3-Mercaptopropyltrimethoxysilane condensates	3-mercaptopropyl		MA	Drug delivery; Biomedicine; Heavy metal binding materials		[73,199,200]
	Dimethicone/mercapto propyl methicone copolymer	3-mercaptopropyl			Hair treatments	Gransil M-SH fluid	[76,77]
	Dimethicone/mercapto propyl methicone and phenyl trimethicone copolymer	3-mercaptopropyl			Hair treatment	Gransil PM-SH fluid	[76,77]
	Poly(dimethylsiloxane)-graft-polyacrylate	Attached ligands: cysteine	Preactivated with 2-mercaptonicotinic acid	MA	Drug delivery		[201]
	Silicon wafers (N-type)	Attached ligands: 1H,1H,2H,2H-perfluorodecanethiol			Antifouling; biomedicine		[202–204]
Proteins	Gelatin	Attached ligands: 4-thiobutylamidine; cysteamine; 3-mercapto-propanoic dihydrazide;4-mercapto butyric acid; N-acetyl-homocysteine	Preactivated with 2-mercaptopyrimidine-4,6-diol	MA; XL	Drug delivery; tissue engineering; regenerative medicine, wound healing	Gelin-S® Hystem® -C Blafar Thiolated Gelatin	[6,205,206]

(continued on next page)

Table 4 (*continued*)

Type of polymer backbone	Polymer backbone	Type of thiolation	Preactivation	Properties	Applications	Products	Representative references (limited to 3 references; for further references please contact the corresponding author)
	Collagen	Attached ligands: 2-mercaptoethylamine; 4-thiobutylamidine; 4-mercapto butyric acid; cysteamine; mercaptosuccinic acid; thioglycolic acid		XL	Regenerative medicine	Gelin-S® Hystem® -C	[207–209]
	Keratin	Reduction with $NaBH_4$	Preactivated with 2-mercaptonicotinic acid		Hair treatment		[210–212]
	Poly(aspartic acid)	Attached ligands: cysteamine; cysteine		XL; MA	Tissue engineering; drug delivery		[22,213,214]
Others	PAMAM dendrimers	N-acetylcysteine; 4-thiobutylaminidne; methyl mercaptoacetate, 3-mercapto propionic acid		PE; EPI; MA	Drug delivery; diagnistics		[215–217]
	PEG	Attached ligands: cysteine; 3-mercapto-propionic acid; thioglycolic acid; tert-butyl mercaptan		XL; MA	Imaging; tissue engineering; wound healing; drug delivery		[218–220]
	PEG-block-poly(L-lysine)	Attached ligands: 4-thiolbutylamidine		XL	Drug delivery		[221]
	Graphene (oxide)	Hydrothermal pyrolysis of carbon source (citric acid) in the presence of reduced-glutathione; Attached ligands: cysteamine		MA	Theranostic nanocarrier; drug delivery		[222–224]
	Poly(glycidol)	Attached ligands: 3-mercapto-propionic acid		XL	Drug delivery; tissue engineering		[48,97,146]
	Polyethyleneimine	Attached ligands: 4-mercapto butyric acid; 4-mercapto butylamidine		MA; XL	Drug delivery; antiperspirant; diagnostics		[26,225,226]
	Poly(aspartamide)	Attached ligands: cysteine; cysteamine; thioglycolic acid			Artificial tear fluid; drug delivery; biomedicine		[65,227,228]
	Poly(vinyl alcohol)	Substitution of hydroxyl groups with thiol groups; Attached ligand: 3-mercapto-propionic acid; cysteine		MA; XL	Drug delivery; Treatment of retinal dispachment; regenerative medicine		[33,229–231]
	Poly(vinylpyrrolidone)	2-mercaptoethyl	Preactivated with 2-mercaptonicotinic acid	MA; XL	Drug delivery		[54]
	Poly(allylamine)	Attached ligands: 4-thiobutylamidine		MA; EPI	Drug delivery		[232–234]
	Graphene oxide	Attached ligands: cysteamine		MA	Drug delivery; diagnostics		[223,235]
	Poly-L-lactide	Attached ligands: cysteine			Drug delivery		[236]
	Polyurethane	Attached ligands: cysteine; thioglycolic acid			Drug delivery		[237,238]
	Polyethylene terephthalate	Attached ligands: cysteine			Drug delivery		[237]

of cysteamine through reductive amination [245]. β-CD-Cys showed 49-fold improved retention time on porcine intestinal mucosa and 35-fold improved retention on porcine buccal mucosa in comparison to unmodified β-cyclodextrin. In another study the ocular residence of α-cyclodextrin was up to 32-fold improved by the immobilization of cysteamine on the oligomeric backbone. As this thiolated cyclodextrin showed no absorption in visible light at all, it was promoted as '*invisible choice to prolong ocular residence time of drugs*' [175].

Further new developments have shown that amphiphilic and even lipophilic polymers such as silicones exhibit mucoadhesive properties through thiolation. Emulsions containing a C10-30 alkyl-modified thiolated carbomer showed almost 10-fold improved mucoadhesive properties on porcine buccal mucosa than emulsions containing the same polymer without thiol groups [187]. Thiolated organosilica nanoparticles from 3-mercaptopropyltrimethoxysilane exhibited a comparatively high ability to adhere to ocular mucosal surface [73]. In another approach the mucoadhesive features of a silicone oil were 3-fold improved by the covalent immobilization of 3-mercaptopropionic acid to this hydrophobic polymer [196].

4.1.2. Adhesion to keratinous surfaces

Keratins are essential for the mechanical stability and integrity of epithelial cells and tissues [246]. They represent also the major component of dry mass in the stratum corneum as well as in the skin appendages such as hair and nails [247]. Due to its high content of cysteine being in the range of 7–20% it is likely an even more attractive target for thiomers than mucin glycoproteins are [248]. Analyses of Griessinger et al. revealed a total amount of 97.8 ± 45.7 μmol/g and 67.8 ± 18.2 μmol/g of free thiol groups available on fresh porcine ear skin and the human horny layer, and a total amount of immobilized thiol groups of 292.1 ± 38.5 μmol/g and 484.0 ± 49.3 μmol/g, respectively [162]. The overall number of ½ cystine in hair keratin corresponds to even 1268–1608 μmol/g [249,250]. On that account, similar interactions between thiolated polymers and the keratin like occurring in mucoadhesion on the base of thiol/disulfide-exchange reactions can be assumed [247,251]. Within an early study adhesive properties of polycarbophil-cysteine on porcine abdominal skin could be verified for the purpose of a more effective transdermal progesterone delivery system [252]. Skin adhesiveness of hydrophilic and lipophilic thiol-modified polymer gels were examined showing that adhesive forces are significantly elevated in comparison to the unmodified control polymers resulting in a 6-fold, 25-fold, 9-fold and 5-fold increase for poly(acrylic acid)-cysteine, Carbopol 971-cysteine, carboxymethyl cellulose-cysteamine and silicone oil thiolated with thioglycolic acid [162]. Partenhauser et al. investigated the potential of attachment of two different sulfhydryl ligands to extend the residence time of poly [dimethylsiloxane-co-(3-aminopropyl)methylsiloxane] on porcine ear skin. After 8 h exposure on skin still 20% of silicone-thioglycolic acid and even 40% of silicone-mercaptopropionic acid remained adherent, whereas unmodified silicon was already completely eliminated under the chosen test conditions [253]. Thi et al. evaluated the adhesive properties of thiolated gelatin hydrogels on porcine skin showing that the more thiol groups are immobilized on the protein the higher are its adhesive properties. In fact, the adhesive properties of their most adhesive thiomer formulation were even 15.8-fold higher than that of a commercially available fibrin glue (Tissucol Duo 500) [206]. Laffleur et al. recorded 7.5-fold and 15-fold higher dermal adhesive forces for S-protected thiolated chitosan and thiolated poly(acrylic acid) derivatives in comparison to the corresponding unmodified polymers [254,255].

Most recently, human hair keratin with an increased number of sulfhydryl groups was S-protected with 2-mercaptonicotinic acid and evaluated for superficial binding to hair fibers in order to reconstitute hair structure. The modification resulted in a higher keratin recovery on hair fibers visualized upon fluorescent marking as illustrated in Fig. 10. In fact, the S-protected highly thiolated keratin was to a 3.6-fold higher extent bound to bleached hair fibers than unmodified keratin [210]. Furthermore, adhesion of thiolated chitosan to keratin of sheep wool was shown to be 2-fold higher than that of unmodified chitosan [256] opening the door for numerous applications especially in textile industry.

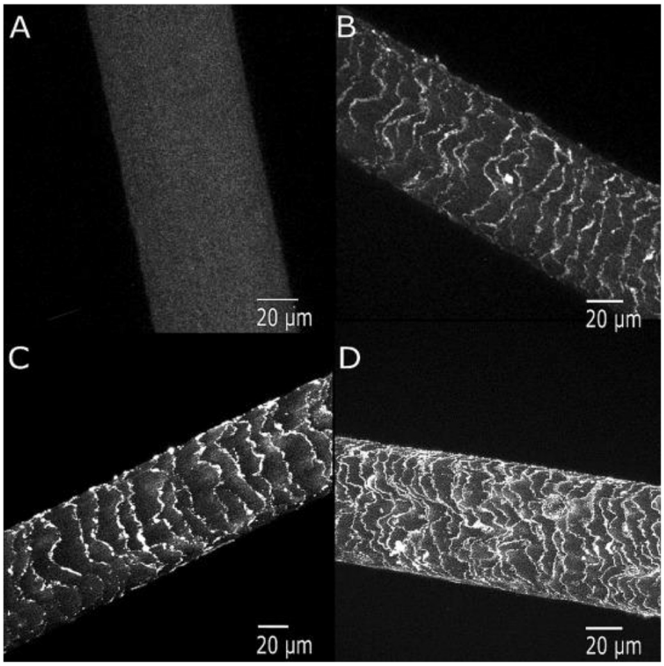


Fig. 10. Fluorescence images of natural hair fibers. Untreated hair fiber (A), fibers with unmodified keratin (B), with highly thiolated keratin (C) and with S-protected highly thiolated keratin (D) bound to the surface [210].

4.1.3. Adhesion to membrane-associated proteins

Apart from mucosal membranes exhibiting cysteine-rich mucins and skin exhibiting cysteine-rich keratins, many cell membrane proteins such as integrins, scavenger receptors, epidermal growth factor receptor or insulin-like-growth factor receptor display cysteine-rich subdomains on the cell surface as illustrated in Fig. 11 [257–259]. Some of these cysteine-rich domains have even been designed by nature to provide adhesion. Integrins, for instance, that are part of the most important cell adhesion mechanism binding and mechanically

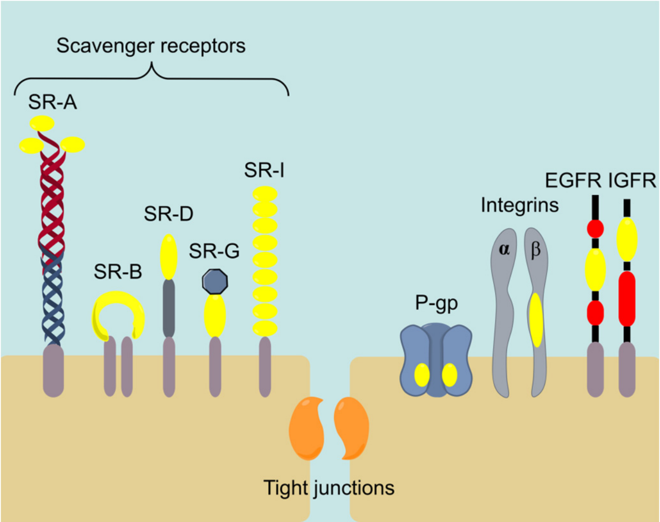


Fig. 11. Overview on membrane-associated proteins with exofacial thiols (highlighted in yellow).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fixing cells to extracellular matrix (ECM), bear a number of cysteine-rich domains with in total 56 cysteine residues on their extracellular β-subunit [259]. These cysteine-rich domains of membrane-associated proteins are interesting targets for thiomers. In addition, various membrane-associated enzymes such as protein tyrosine phosphatase exhibit a thiol substructure in their active center [260], making them to interesting targets as well. These exofacial thiols seem to be involved in various mechanisms such as cell uptake [261,262]. Aubry et al., for instance, could find for a model peptide exhibiting a cysteine substructure that was bound to another cysteine via a disulfide bond almost no cellular uptake. In contrast, when this cysteine substructure on the peptide was activated with 3-nitro-2-pyridinesulfenyl to form new disulfide bonds with exofacial thiols, the peptide was to a high extent transported inside the cells [263]. Furthermore, exofacial thiols were shown to be involved in the opening of tight junctions [260] [257] or the inhibition of efflux pumps such as P-glycoprotein (P-gp) [264]. Thiomers exhibiting permeation enhancing properties and efflux pump inhibiting properties are listed in Table 4. As certain cancer cells were shown to express a comparatively higher level of exofacial thiols [265], these functional groups might provide a promising anchor for targeted drug delivery. As thiomers are capable of forming not just one but several disulfide bonds with membrane-associated proteins, they are likely more tightly bound than monothiols. Although the binding and interaction of thiomers to exofacial thiols has already been shown in various studies, it has to be pointed out that most of these studies have been largely limited to immortalized cell lines or rodents. More recently Glass et al. provided evidence for the enhanced association of thiomers to distinct human blood cells providing more valid data regarding the potential of thiomers to adhere to membrane-associated proteins [266]. Moreover, a stimuli-responsive system for controlled cell adhesion and detachment made of thiolated chitosan and thiolated chondroitin sulfate was constructed. Owing to the formation and cleavage of inherent disulfide bonds by oxidation and reduction, surface properties of the system were controlled toward human fibroblasts adhesion in a reversible manner. The system might be useful for biomedical applications as responsive and obedient surfaces in medical implants [57]. Gevrek et al. established a similar 'catch and release' hydrogel platform for cells based upon thiol-disulfide exchange reactions [267].

4.2. In situ gelation

In situ gelling properties are advantageous for numerous applications in life sciences. In drug delivery the efficacy of many topical liquid formulations such as ocular, nasal and vaginal formulations can be improved, as the sol-gel transformation after application prevents from a rapid outflow and provides prolonged residence time [268]. Such formulations can be administered as liquids forming in the following stable gels on the target mucosa. Furthermore, *in situ* gelling properties play a vital role for injectable hydrogels and for hydrogels used as scaffold matrices that are widely used in tissue engineering [269]. Apart from that, *in situ* gelling properties are also of practical relevance for various further applications like for coating materials [270] or for food additives [271]. Thiomers as listed in Table 4 were shown to exhibit such *in situ* gelling properties.

4.2.1. In situ gelation via oxidation and thiol/disulfide exchange reactions

Thiolated polymers exhibit comparatively high *in situ* gelling properties because of inter- and intrachain disulfide crosslinking due to oxidation. As the oxidation process depends on the amount of thiolate anions on each polymer, crosslinking can be controlled by the quantity and pKa value of thiol substructures on the polymer and pH. The *in situ* gelling properties of thiolated polymers, for example, were shown to increase with an increasing degree of thiolation [20,272]. The process of disulfide crosslinking can be accelerated in the presence of oxidizing agents like hydrogen peroxide and periodate or enzymes such as peroxidase [81,206]. For thiolated chitosan a 10,000-fold increase in viscosity could be achieved within minutes due to the addition of hydrogen peroxide [81]. In another study an even 100,000-fold increase in viscosity within an hour was achieved for the same type of thiomer due to addition of carbamide peroxide [7]. Furthermore, oxidized glutathione was shown to trigger *in situ* gelation of thiomers. In contrast to oxidizing agents described above, it mediates the crosslinking of thiomers via thiol-disulfide exchange reactions being thereby reduced to the monomer. Zarembinski et al. could determine gelation times for thiolated hyaluronic acid and thiolated gelatin hydrogels of less than 5 min due to the addition of oxidized glutathione in a concentration of 2-10 mM [113]. In particular for injectable thiomer hydrogels, oxidized glutathione seems to be advantageous because of its history of safe use in humans. Hydrogels having been crosslinked with oxidized glutathione exhibited promotion of 3D culture of stem cells and biocompatibility in intracutaneous and subconjunctival *in vivo* investigations using white rabbits [113,273].

Entirely S-protected thiomers offer the additional advantage that they do not crosslink at all during storage even in form of aqueous liquids. Similar to an oxidized glutathione mediated *in situ* gelation their crosslinking can also be initiated via thiol/disulfide exchange reactions. Getting into contact with free thiols of mucins, keratins or ECM proteins the crosslinking process is initiated exactly there where it is supposed to take place. Fürst et al., for instance, showed that an entirely S-protected silicone increases its viscosity only due to the addition of N-acetylcysteine having been used as representative thiol bearing compound [201].

4.2.2. In situ gelation via thiol-ene reactions

In case of thiol-ene reactions the crosslinking can be controlled by the type and amount of added alkenes. As listed in Table 3 there are different types of alkenes exhibiting two, three and four ene substructures available. Thiolated gelatin, for instance, was crosslinked with poly(ethylene glycol)diacrylate through thiol-ene reaction providing a scaffold of tunable mechanical features supporting cell attachment and growth [6]. In addition, thiomers being crosslinked with most of such alkenes do not seem to bear any toxicological risks. To address spinal cord injuries, an injectable *in situ* gelling system based on thiolated chitosan was crosslinked with a four-arm polyethylene glycol terminated in maleimides. Injected into the spinal cord of rats for two weeks no host reactions at all were observed [274]. Furthermore, even polymers displaying multi ene substructures were already used to crosslink with thiomers. Russo et al. crosslinked thiolated gelatin with pentenoyl-modified gelatin via thiol-ene reaction and obtained stable hydrogels mimicking ECM structure [275]. Wang et al. designed thiol- and vinyl-modified poly(N-isopropylacrylamide) copolymers possessing physical gelation properties on the one hand deriving from hydrophobic aggregation of the polymers and on the other hand by chemical crosslinking via Michael addition reaction of thiol- and vinyl substructures [276]. Another example is given by a mixture of thiolated hyaluronic acid and a (meth)acrylate copolymer [277].

4.3. Controlled drug release

4.3.1. Diffusion-controlled drug release

Drug release controlling properties are of high importance in drug delivery. Because of a sustained drug release a sufficiently high therapeutic drug level on the target tissue in case of local treatments or in blood in case of systemic drug delivery can be maintained over a prolonged period of time. The frequency of dosing can be consequently reduced. Disulfide bond formation under swelling of the thiolated polymers generates a three dimensional network from which incorporated drugs can be released over several hours in a diffusion-controlled manner. In this way, limited efficacy of polymeric drug delivery systems due to rapid disintegration or erosion of the polymeric matrix can be overcome. Polycarbophil matrix tablets were shown to disintegrate within 1.5 hours, whereas thiolated polycarbophil matrix tablets remain stable for at least 48 hours providing a sustained drug release [278]. Making use of this release controlling effect, Hornof et al. showed in a clinical trial a sustained release of sodium fluorescein from thiolated polyacrylate matrix tablets used as ocular inserts over 8 hours on the ocular surface. In contrast matrix tablets comprising just the polyacrylate did not provide a sustained drug release at all [279]. Similar ocular delivery systems were developed by Jade Therapeutics Inc. for antibiotics utilizing thiolated hyaluronic acid [280]. The release controlling effect achieved by disulfide bond formation within polymeric drug carrier systems was also shown for hydrogels and nanoparticles. Moreno et al. prepared hydrogels comprising of S-protected thiolated chitosan and investigated the impact of crosslinking on the release of ranibizumab and aflibercept [281]. Higher crosslinking density and less swelling capacity resulted in lower burst release. Thiolated chitosan was further utilized in particles to decelerate the release of curcumin and 5-fluorouracil into the blood. An anticancer study with these particles using colon cancer HT29-cells revealed up to 3-fold superior efficacy than the control [282]. Furthermore, blood circulation time and distribution of 5-fluorouracil to the lungs could be substantially increased by utilizing cysteine modified poly-L-lactide/Pluronic F-68 particles [236].

4.3.2. Reduction-controlled drug release

Another release controlling mechanism of thiomers making use of the 3D network formed by inter- and intrachain disulfides is utilizing the bioreducible properties of these bonds [283]. Kataoka and co-workers were likely the first describing a bioreducible delivery system [284]. Based on the existing concentration gradient of glutathione (GSH) between the extra- and intracellular compartment (GSH intracellular: ~1-11 mM and extracellular: 1.5 μM), disulfide bonds within delivery systems are cleaved in the glutathione-rich environment of the cytosol releasing selectively the cargo [285,286]. Fig. 12 schematically pictures the principle of intracellular delivery using bioreducible polymers. In particular for intracellular delivery of genes, short interfering RNA (siRNA) and chemotherapeutics this release mechanism is of relevance [287–289]. Carrier systems comprising multiple functional groups such as amines and disulfide bonds exhibit high DNA condensation ability combined with a rapid degradation under reducing conditions. A large variety of polymers containing disulfide bonds have been examined within the scope of gene delivery such as poly(amido amines), poly(amino esters), oligomerized amines, copolymers of methacrylic acid, chitosan and hyaluronic acid based nanogels among others. Poly(amido ethylenimine) polymeric nanoparticles with multiple disulfide bonds as carrier for pDNA initiated nearly 20-fold higher transfection than the poly(ethyleneimine) control in different types of cells [290]. Within another study of the same group a 4-fold higher vascular endothelial growth factor (VEGF) protein expression versus control could be achieved in the region of infarct by using a disulfide poly(amido ethylenediamine) carrier in rabbits [291]. McGinn et al. achieved successful VEGF transfection of myoblasts with poly(cystaminebisacrylamide-diaminohexane) in rats engendering induction of neovascularization, reduction of cardiac apoptosis and restoration of ventricular function [292]. Arginine-grafted poly(disulfide amine) polymer particles could demonstrate their effectiveness in delivery of siRNA in a mouse tumor model [293]. Yin et al. evoked a synergistic anti-tumor effect of paclitaxel and siRNA by using a system comprising an octyl-modified disulfide containing polyethylenimine core and a thiolated hyaluronic acid anionic shell [225]. The hyaluronic acid shell enabled on the one hand CD44-mediated active targeting and supported on the other hand the co-burst release of the active ingredient in the cytoplasm as it is degraded by hyaluronidase. *In vivo* studies confirmed significantly higher tumor growth suppression than under application of commercially available Taxol®. Core shell micelles stabilized by aerial oxidation of mercapto groups into disulfides represent a second innovative type of carriers in regards of intracellular delivery [294–296]. Micelles containing thiolated poly(ethylene glycol)-block-poly(L-lysine) provoked a 100-fold higher siRNA transfection efficacy in comparison to a control formulation [221].

4.3.3. Controlled release of covalently attached drugs

Drugs with thiol substructure such as in particular peptide and protein drugs and various anticancer drugs can be covalently bound to thiomers via disulfide bonds and released in a controlled and sustained manner via thiol/disulfide exchange reactions or in a reductive environment. The potential of this delivery technology has meanwhile been shown in numerous *in vitro* and *in vivo* experiments [66,289,297,298] [289,297,299–301]. Chen et al., for instance, bound a therapeutic

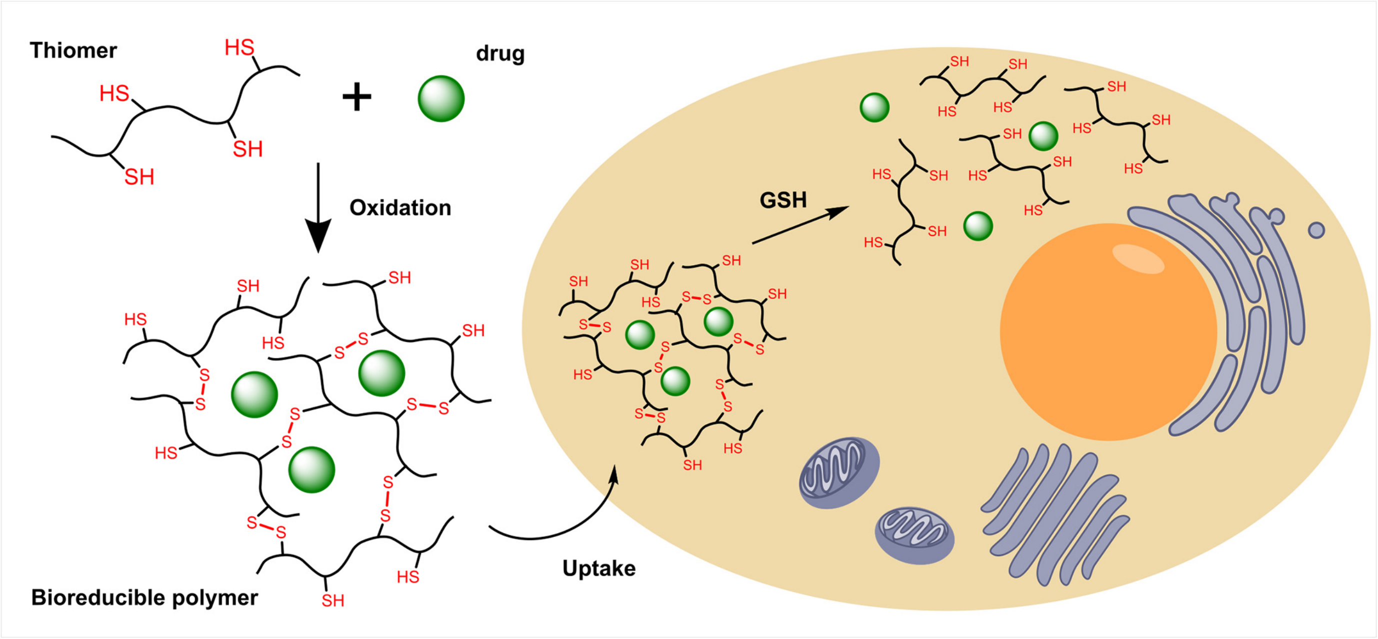


Fig. 12. Schematic illustration of the principle of drug delivery with thiomers; reduction of disulfide bonds of the carrier mediated by glutathione in the cytosol and release of the incorporated drug.

peptide to a thiolated chitosan via disulfide bonds. *In vitro*, a sustained release of the bioactive peptide for up to 90 days was achieved and *in vivo* the superior performance of this delivery system was confirmed by the repair of bone defects [301]. In another study a peptide drug was covalently attached to thiolated carboxymethyl dextran resulting in 5-fold extended elimination half-life, 3-fold decreased volume of distribution and 6.7-fold decreased plasma clearance rate compared to the peptide being injected in unconjugated form [168]. Thiolated hyaluronic acid was used for time-controlled release of proteins [302]. Zhuang et al. bound the anticancer drug 6-mercaptopurine via disulfide bonds to a thiomer self-assembling into amphiphilic micelles in aqueous media. Under the reductive conditions in the cytosol of tumor cells the drug was released in a targeted manner [72].

Soon after the introduction of thiomers, they were also adopted as promising tool to provide a sustained release of nitric oxide (NO) as they can be easily nitrosated and are reusable due to possible renitrosation. As NO supports several functions in the body such as blood flow regulation, inhibition of platelet adhesion and aggregation and smooth muscle relaxation, its delivery is of high relevance to treat deficits of intrinsic NO. Besides treatment of blood circulation disorders and thrombosis, NO owns antimicrobial activity making it also a useful tool for treatment of bacterial infections [303,304]. Due to the inhibition of bacterial adhesion and proliferation, platelet adhesion and aggregation, NO releasing systems are furthermore promising for use in regenerative medicine preventing possible inflammatory responses [129]. Duan and Lewis were likely the first describing a catalyzed NO release from polyurethane and polyethylene terephthalate having been thiolated with L-cysteine [237]. Both thiomers decreased platelet adhesion by more than 50% compared to the corresponding non-thiolated polymers. Fumed silica polymer filler particles with cysteine, N-acetylcysteine or N-acetylpenicillamine substructures showed also a sustained NO release [305]. In another study NO release from thiolated polyurethanes was optimized by screening sulfhydryl ligands of different chain length for their suitability [238]. A thiolated PEG subsequently modified with NO exerted *in vivo* a prolongation of blood pressure reduction. Due to a significantly deaccelerated NO release, there was a 1.7–fold improved effect in mice and up to 3.7-times in rats compared to common S-nitrosothiols [306]. Enabling an increased NO storage, dendrimers were also identified as promising NO delivery vehicles. Thiolated NO-dendrimers were capable to store and release NO up to 2100 μmol per gram preventing platelet aggregation to a higher extent than the corresponding small molecule NO donor [307]. Furthermore, thiolated alginate and thiolated dextran were already successfully adapted for controlled NO release [129,308].

4.4. Permeation enhancement

Thiolated polymers were shown to reversibly open tight junctions (TJs). This permeation enhancing effect is shown in numerous *in vitro* and *in vivo* experiments (Table 4). The responsible mechanism is likely based on their interaction with thiol substructures of certain enzymes and membrane bound proteins being responsible for TJs opening [260,257]. Clausen et al. suggested a thiomer mediated inhibition of protein tyrosine phosphatase dephosphorylating tyrosine subunits on occludin causing TJs opening [260]. A recent study suggested an additional mechanism as illustrated in Fig. 13. By interacting with highly expressed cysteine-rich membrane receptors like insulin-like growth factor receptor (IGFR) and epidermal growth factor receptor (EGFR) thiomers activate proto-oncogene tyrosine-protein kinase (Src) phosphorylation and further disrupt claudin-4 resulting in TJs opening [257]. In contrast to many other permeation enhancers, this TJs opening through thiomers was shown to be reversible [216]. The permeation enhancing properties of polymers like chitosan can be even 40-fold improved by thiolation [309]. This permeation enhancing effect is more pronounced than that of most other permeation enhancers. Thiolated polycarbophil, for instance, exhibits a higher permeation enhancing effect than sodium caprate applied in the same concentration representing one of the gold standards for oral permeation enhancers [186]. Moreover, the permeation enhancing properties of S-protected thiolated polymers are higher than that of free thiomers [23,257,310,311]. In contrast to low molecular mass permeation enhancers, thiomers cannot be absorbed from mucosal membranes because of their high molecular mass. Hence, their permeation enhancing effect remains concentrated on the absorption membrane and systemic toxic side effects can be avoided. As the permeation enhancing effect of thiolated polymers is based on a completely different mechanism than that of all other enhancers, the combination of thiomers with other permeation enhancers should result in an additive effect. Although it is challenging to determine the permeation enhancing effect of thiomers *in vivo* as various other effects such as their mucoadhesive and enzyme inhibitory properties contribute to an improved bioavailability, evidence for their potential as permeation enhancers on different mucosal tissues like intraoral, intestinal and nasal mucosa could be provided in both rodent and non-rodent animal models e.g. [14,114,186,312–315]. The oral absorption of insulin, for example, was even 20-fold improved by the co-administration of a thiolated chitosan instead of unmodified chitosan [14]. Results of this study are illustrated in Fig. 14.

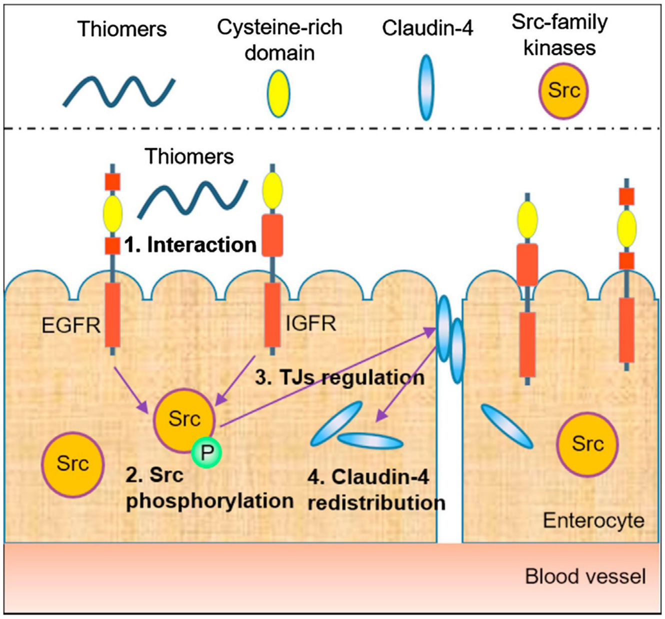


Fig. 13. Illustration of the postulated mechanism being responsible for the opening of tight junctions (TJs) through thiomers; by interacting with the highly expressed cysteine-rich membrane receptors epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor (IGFR) thiomers activate proto-oncogene tyrosine-protein kinase (Src) phosphorylation and further disrupt claudin-4 resulting in tight junctions opening; adopted from Zhang et al. [257].

4.5. Efflux pump inhibition

The mucosal absorption of numerous therapeutic agents including antiinflammatory, anticancer and antimycotic drugs is limited by efflux pumps such as multidrug-resistance protein and P-gp [316,317]. Their inhibition is therefore an important strategy to improve bioavailability of these drugs. Thiolated polymers were shown to inhibit efflux pumps in a reversible manner and this effect seems to be more pronounced than that of other polymeric efflux pump inhibitors [116,216,318,319]. Föger et al. compared different polymeric efflux pump inhibitors using rhodamine 123 as substrate for P-gp demonstrating in rats the superior effect of the tested thiolated polymer [319]. The underlying mechanism for this inhibition seems to be based on the

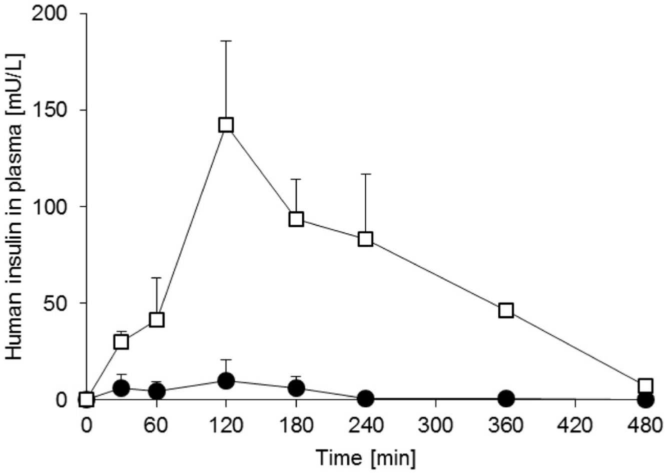


Fig. 14. Plasma concentration after oral administration of insulin/thiolated chitosan tablets (□) and insulin/unmodified chitosan tablets (●) to rats; adopted from Millotti et al. 2014 [14].

formation of disulfide bonds of thiomers with cysteine substructures being located in the channel of these pumps [264]. Due to the blockage of these channels the mucosal uptake of drugs that are substrates of efflux pumps can be essentially improved [216,319,320]. By the co-administration of thiolated xyloglucan oral bioavailability of the P-gp substrate lopinavir was 3-fold improved [155]. The combination of chitosan-thiobutylamidine with glutathione enhanced the oral bioavailability of rhodamine 123 4.3-fold [321,322]. Furthermore, in cancer induced rats paclitaxel plasma levels were significantly improved by the co-administration of thiolated polycarbophil accompanied by a substantial reduction in tumor growth [323]. At physiological pH conditions alkyl thiols fail in reaching full reactivity as they possess a pka value of 8-10. Thiol protection by coupling of thiolated pyridyl substructures, however, leads to pH independent reactivity. As a consequence, disulfide bond formation between S-protected thiol groups and cysteine containing transmembrane domains is more efficient. Netsomboon et al. investigated the inhibitory potency of a 2-mercaptonicotinic acid S-protected poly(acrylic acid)-cysteine derivative (PAA-Cys-2MNA) in a Caco-2 cell model [182]. The permeation of rhodamine 123 was 2.36-fold higher in the presence of PAA-Cys-MNA. Comparable values of apparent permeability coefficient (Papp) to rhodamine 123 alone were obtained after wash-off of the thiomer confirming the reversibility of inhibition [324].

4.6. Metal binding properties

Thiols are generally known for their metal ion binding properties. Penicillamine and 2,3-dimercapto-1-propanol, for instance, are used because of their copper(I)-binding properties for the treatment of Wilson disease [325]. Thiolated polymers can exhibit comparatively strong and fast metal ion binding properties. As many proteases such as chymotrypsin need zinc ions as co-factor, thiolated polyacrylates were shown to inhibit this enzyme [326]. Oral administration of peptides, proteins or nucleic acids is challenging because the enzymatic barrier within the gastrointestinal tract needs to be faced and overcome before the onset of the desired therapeutic effect. Most of the proteolytic enzymes require the presence of divalent cations as cofactors to maintain their structural integrity as a prerequisite for enzymatic activity. Deprivation of calcium provokes the inhibition of the Ca^{2+}-dependent serine proteases trypsin and α-chymotrypsin [327] and the reduced activity of carboxypeptidase A and cytosolic leucine aminopeptidase is directly related to the extent of Zn^{2+} deficiency [328]. Co-administration of thiolated polymers proved to be a valuable strategy to improve bioavailability of peptide therapeutics by lowering their presystemic metabolism. As an example aminopeptidase N could be successfully inhibited on intestinal and vaginal mucosa by thiolated poly(acrylates) [329] and thiolated polycarbophil [330]. Reversible binding of cations by the thiol group functionality could be verified as the underlying mechanism of inhibition. Bravo-Osuna et al. reported about positive effects on calcium binding capacity of thiolated poly(isobutyl cyanoacrylate) core-shell nanoparticles [331]. The sulfhydryl group has also a high affinity for heavy metals. Thiomers bind heavy metals such as mercury, lead, gold and cadmium tightly. The word mercapto is derived from the Latin *mercurium captāns* as thiols are known to capture mercury. Thiolated chitosans were shown to efficiently bind heavy metals such as Hg (II), As (III) and As(V) [37] [332]. Furthermore, thiolated polymers can efficiently improve the stability of aqueous dispersions of gold carriers by forming a protective shell under covalent immobilization of the thiol moiety to the gold surface [333–337].

5. Applications of thiolated polymers

These unique properties of thiomers resulted in numerous applications and product developments in various fields. The likely most advanced and most promising developments are highlighted in the following.

5.1. Drugs

Generally, mucoadhesive polymers show a beneficial effect in treatment of disorders where dry mucosal surfaces are involved. In particular in case of dry mouth, dry eye and dry vagina syndrome also known as Sjögren syndrome lubricating mucoadhesive polymers are a potent treatment option to restore physiological conditions by substituting the missing mucus. As a consequence, they are able to improve tear-film break-up time, alleviate chewing difficulties and ease local irritation or itching. Because of their comparatively much higher mucoadhesive and *in situ* gelling properties, thiomers are more efficient than the corresponding non-thiolated polymers [338]. They actually mimic mucus glycoproteins being secreted on mucosal membranes in reduced form anchoring thereafter within the mucus layer via disulfide bond formation.

An Austrian company developed eye drops containing chitosan–N-acetylcysteine called Lacrimera® [339,340]. In one of their clinical trials the difference between treated and untreated eye of healthy volunteers treated for five consecutive days with one drop of 0.1%, 0.2 % or 0.3% thiomer was evaluated [341]. Results of this trial revealed a significant improvement in ocular surface disease index (OSDI) for 83% of test persons without any side effects of the thiolated polymer [342,343] [344]. In addition, single-dose instillation of Lacrimera® for several days improved in another clinical trial symptoms and signs in patients suffering from dry eye syndrome, who were refractory to treatments with well-established lubricants [344]. In a long-term clinical trial over 26 weeks in patients suffering from dry eye the OSDI improved almost 4-fold by just once-daily use of the thiolated chitosan. Results are illustrated in Fig. 15 [345]. Furthermore, local tolerability of Lacrimera® was examined in healthy young volunteers with or without use of contact lenses [102,346]. In the most recent trial the corneal residence time was detected and compared with placebo [347]. Results show safety and efficacy of a single dose making this treatment a promising alternative to up to now multiple dose therapies [341]. The product even won the German Innovation Award 2018 [348].

Thiolated polymers like thiolated poly(aspartamide) and thiolated hyaluronan are in the pipeline for the same indication [227,349]. Clinical trials are listed in Table 5. Thiolated carboxymethyl hyaluronic acid was compared to a standard hyaluronic acid-containing tear supplement in a randomized, masked clinical study in dogs affected from keratoconjunctivitis sicca. The thiomer significantly (p = 0.0003) improved ocular surface health in comparison to alternative lubricants [349]. These

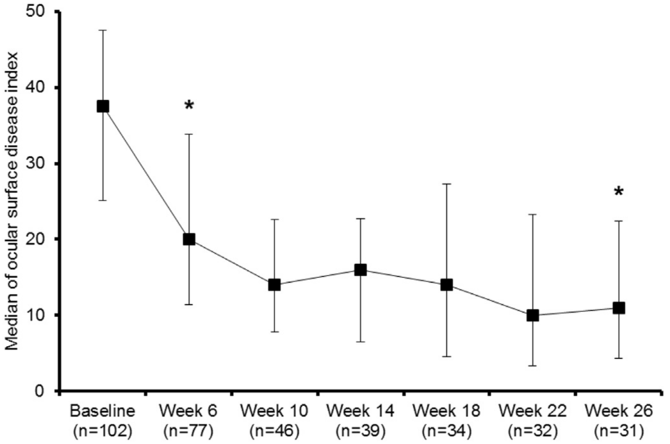


Fig. 15. Ocular surface disease index (OSDI) during treatment with chitosan-N-acetylcysteine eye drops; adopted from Lorenz et al. [345].

results were in the following confirmed by a clinical trial in 20 Sjögren syndrome patients showing higher efficacy of thiolated hyaluronic acid compared with unmodified hyaluronic in maintaining the stability of the tear film [350].

Because of their *in situ* gelling properties thiomers are also a promising tool for various surgical procedures. Partenhauser et al., for instance, could show the advantage of S-protected thiolated silicones for long-term vitreous replacement. Thiomers could be aspired with a 20 G needle exhibiting a reversible sol-gel transition. The percentage of emulsification demanding premature termination of the treatment was in case of these thiolated silicones below 8%, whereas the corresponding unmodified silicone was already 100% emulsified [351]. Moreover, a clinical trial demonstrated safety and efficacy of thiolated hyaluronan when implanted into the ocular bulb to treat primary open angle glaucoma [352]. Recently, Eyegate Pharmaceuticals Inc. developed a bandage gel containing thiolated carboxymethylated hyaluronic acid supporting tissue regeneration after ocular surgery verified in a clinical trial comprising 39 patients [353]. Thiomer hydrogel alone exhibited potential as human demineralized bone matrix indicating complete filling with regenerated native bone after injection into 5 mm defects in bone tissue [354]. Additional incorporation of bone morphogenetic protein 2 led to an increased osteoblastic cell differentiation accompanying a 1.8 fold bone formation due to a prolonged release [355].

Another property of thiomers that has attracted attention is their use as antioxidants and oxidizing free radicals scavengers [356]. Oxidizing free radicals play a crucial role contributing to the onset and progression of inflammation. In early stages free radicals lead to the expression of

Table 5
Overview about studies in rabbits, pigs, dogs, horses and humans.

Thiomer	Application	Species	Results	References
Chitosan-N-acetylcysteine	Drug delivery	Rabbits	Ocular clearance of nanocarriers coated with chitosan-NAC was significantly delayed and the effect was positively related to the degree of thiolation.	[47]
	Dry eye syndrome		An aqueous solution of Na^{124}I was rapidly cleared from the eye, whereas ^{124}I-labelled chitosan-NAC remained on the ocular surface for 22 hours.	[101]
	Wound healing		Twice daily instillation of eye drops containing chitosan-NAC caused a faster corneal wound healing than a placebo ($p < 0.01$).	[387]
Thiolated carboxymethyl hyaluronic acid	Wound healing		Significantly improved corneal wound healing	[388]
			Thiolated hyaluronic acid films facilitate re-epithelialization and decrease the area of corneal opacity in a corneal alkali burn rabbit model	[111]
			Enhanced survival and morphology of corneal cells	[389]
	Drug delivery		Sustained intraocular release of a peptide drug	[113]
Thiolated hyaluronic acid	Retinal detachment repair		Retinal detachment repair superior to silicone	[390]
	Wound healing		Healing of cartilage defects	[391]
Thiolated poly(acrylamide) copolymers	Vitreous substitute		The reduced thiomer hydrogel could be easily injected into the vitreous cavity and underwent rapid gelation within the eye.	[55]
Thiolated poly(amido ethylenediamine)	Drug delivery		Efficient non-viral gene delivery mediating high levels of gene expression *in vivo*	[291]
Chitosan-4-thio-butylamidine conjugate	Mucoadhesive and permeation enhancing drug delivery system	Pigs	3.2% oral bioavailability (vs. sc) of the peptide drug antide; unformulated antide did not show any systemic uptake at all;	[114]
			1% intraoral bioavailability (vs. iv) of the peptide drug PACAP; unformulated PACAP did not show any systemic uptake at all;	[315]
Thiolated gelatin	Sensor		Improved sensitivity of glucose sensors	[386]
Thiolated carboxymethyl hyaluronic acid	Dry eye syndrome	Cats	Significantly accelerated closure of acute corneal stromal ulcers	[392]
	Dry eye syndrome	Dogs	Significantly reduced symptoms of dry eye syndrome within two weeks while being applied twice daily; significantly accelerated closure of acute corneal stromal ulcers	[112,349,392]
	Wound healing		Enhanced rate of wound healing	[393]
		Horses		[393]
Thiolated gelatin	Osteoconductive device		The thiolated polymer appeared to be a biocompatible orthopaedic implant	[394]
Chitosan-N-acetylcysteine	Treatment of dry eye syndrome	Humans (clinical trials)	In patients with dry eye syndrome single administration of eye drops containing chitosan-NAC significantly increased the mean tear film thickness; OSDI was 4-fold improved;	[102,346,395,341,342,347,396,345]
Chitosan-cysteine	Mucoadhesive drug delivery system		Prolonged intraoral residence time of the chitosan-cysteine drug delivery system	[367]
Thiolated carboxymethyl hyaluronic acid	Dry eye syndrome		Tear film stability in Sjögren syndrome patients was significantly improved	[350]
Thiolated hyaluronic acid	Intraocular implant		Safety of thiolated hyaluronic acid as intraocular implant was shown	[397]
Thiolated hyaluronic acid	Reepithelialization		An ocular bandage gel containing thiolated hyaluronic acid accelerated reepithelialization after photorefractive keratectomy	[353]
Thiolated polyacrylic acid	Mucoadhesive drug delivery system		Prolonged ocular residence time of a thiolated vs. non-thiolated drug delivery system	[279]
Thiolated alginate	Mucoadhesive drug delivery system		Enhanced mucoadhesive and drug release controlling features were shown for an intraoral metformin delivery system	[368]

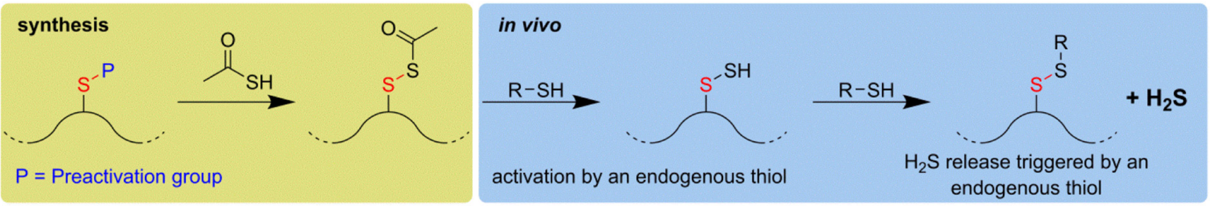


Fig. 16. Synthetic pathway for the transformation of S-protected thiomers to *per*-thiomers according to Prinz and Hoffer [361] and hydrogen disulfide release from *per*-thiomers mediated by endogenous thiols *in vivo*.

cytokines. In the following they activate together with cytokines endothelial cells and cause toxic effects by inducing loss of cell function. As there is a broad acceptance of the equation oxidative stress = inflammation, and glutathione is regarded as an endogenous anti-inflammatory mediator [357], thiolated polymers should consequently show anti-inflammatory properties. Thiomer nanoparticles, for instance, were shown to downregulate TGFβ1 being induced by interleukin-6 making the thiomer to a promising tool for treatment of corneal haze [358]. In another study the anti-inflammatory effect of (thiolated) chitosan nanocarriers containing theophylline against allergic lung inflammation was evaluated in mice. Although neither blank chitosan nor blank thiolated chitosan nanocarriers showed anti-inflammatory properties, the effect of theophylline was significantly enhanced when being delivered with thiolated chitosan nanoparticles [359].

Hydrogen sulfide is of high therapeutic relevance as cellular signaling agent playing a critical role in various pathological processes. It is believed to be involved in angiogenesis, inflammation and apoptosis [360]. Although there are numerous hydrogen sulfide releasing compounds available, they cannot provide a controlled hydrogen sulfide release. More recently, thiomers were designed in a way that they slowly release low amounts hydrogen sulfide. Polymers with acyl-protected perthiol substructures can be obtained by thiol/disulfide exchange reaction of thiomers having been S-protected with thioamides such as mercaptopyridine or mercaptonicotinic acid with thioacids such as thioacetic acid or thiobenzoic acid [361]. The synthetic pathway is illustrated in Fig. 16. Such acyl-protected *per*-thiomers were shown to release hydrogen disulfide when exposed to L-cysteine in a controlled manner [362,363]. Taking also the high bioadhesive properties of thiomers into account not just a controlled but also a targeted release of hydrogen sulfide at diseased tissue seems feasible.

5.2. Drug delivery systems

Because of their mucoadhesive properties thiolated polymers can prolong the mucosal residence time of various types of drug delivery systems. Consequently, the local therapeutic effect of APIs is prolonged and for a systemic drug delivery more time for drug absorption from mucosal membranes is available. Moreover, their drug release controlling, *in situ* gelling, permeation enhancing, efflux pump inhibiting and enzyme inhibiting features make them to a versatile and powerful tool for drug delivery. This great versatility and flexibility of thiomers allowing the most suitable design for each drug and delivery purpose, however, is from the industrial point of view disadvantageous. Because of the high investments for the development of new excipients and the long time needed for the development process being in the range of that for new drugs, much less new excipients than new drugs are getting registered each year. In order to justify these costly and time-consuming developments, new excipients have consequently to show a very broad applicability for numerous drugs. The identification of certain thiomers, that can address an as broad as feasible range of industrial drug delivery problems, will therefore be key to their success in drug delivery. As the benefits of thiomers for drug delivery have been shown in hundreds of research articles as summarized in numerous reviews e.g. [25,103,105,106,364–366], we focus herein just on results of clinical trials providing insights in their overall performance.

Samprasit et al. developed mucoadhesive electrospun nanofibre mats containing an antimicrobial API using chitosan and thiolated chitosan as mucoadhesive polysaccharide. The therapeutic effect and mucoadhesion were determined in healthy human volunteers. Thiolated chitosan formulations showed higher mucoadhesive properties than those containing the corresponding unmodified polymer. Results demonstrated that thiolated chitosan mats have the potential to be mucoadhesive dosage forms to maintain oral hygiene through reduction of bacterial growth causing caries [367]. Furthermore, clinical studies on 20 subjects using thiolated alginate as mucoadhesive film to deliver metformin for periodontal diseases were performed. Films composed of 6% carboxymethyl cellulose and 4% thiolated alginate demonstrated strong mucoadhesion, notable swelling and sufficient drug release over 12 h resulting in a nonsurgical treatment of chronic periodontitis [368,369]. The study provides strong evidence for the potential of thiomers as superior vehicles for mucosal drug delivery.

Moreover, clinical studies in healthy volunteers investigated the effect of thiolated polyacrylic acid on ocular residence time of a model API. Results demonstrated that a constantly high drug concentration in the tear fluid can be maintained with the thiomer for numerous hours, whereas the drug was eliminated during the first hour when the corresponding un-thiolated polymer was used. T_{max} was 384 min for the thiolated polyacrylate formulation, whereas it was 20 min for the unmodified polyacrylate formulation. The thiolated polyacrylate was well-tolerated on the ocular surface [279]. The study provides strong evidence for the high mucoadhesive and cohesive features of thiolated polymers and the ability to control drug release out of such systems over numerous hours.

5.3. Diagnostics

Gold nanoparticles are used for biomedical applications in disease diagnostics. Unique optical and physical properties such as non-cytotoxicity, biocompatibility, ease of preparation, surface functionalization, strong light absorption, scattering effect, high photothermal conversion rate and photostability [370] enable bioimaging [371] such as ultrasonic, photoacoustic, computed tomography and thermal imaging as well as selective targeting [372–375]. One major weakness of gold nanoparticles, however, is their tendency to aggregate in aqueous dispersions, especially in the presence of high salt concentrations or in contact with endogenous proteins. The likely most efficient way to overcome this problem is the coating of gold nanoparticles with thiolated polymers. Already a few years after their discovery first polymers exhibiting at least one thiol group came therefore into use as coating material for gold nanoparticles [376]. The thiomer gold coating offers also the possibility of conjugating targeting ligands to other functional groups on the polymer. Chattaway et al. generated a dual- functionalized surface by coupling a thiolactone-based copolymer consisting of N,N-dimethylacrylamide and thiolactone acrylamide via aminolysis in the presence of an amine derivative to gold [377].

Selective staining of pancreatic tumor stroma was attained by binding of antibody-conjugated PEGylated nanoparticles [378]. Paciotti et al. examined PEGylated gold nanoparticles for the targeted delivery of tumor necrosis factor (TNF) to a solid tumor in mice. Thiol-derivatized PEG and recombinant human TNF were immobilized on the gold surface through disulfide bonds to obtain PEGylated-gold-TNF nanoparticles. Upon intravenous administration, TNF anchored the nanoparticles mainly in colon carcinoma tumors while binding to its receptor [379].

Apart from gold nanoparticles iron oxide-gold core-shell nanoparticles were successfully coated with thiolated alginate [380] and with thiolated polyethyleneimine [226], exhibiting excellent colloidal stability and comparatively high cytocompatibility. Furthermore, aqueous dispersions of core-shell nanoparticles comprising of a magnetic iron oxide core coated with gold were stabilized by thiolated pectin providing storage stability for several weeks [381]. Due to co-existence of iron oxide and gold these nanoparticles can be used for magnetic resonance and computed tomography imaging of tumors.

Moreover, iron oxide nanoparticles were also directly coated with thiomers such as thiolated polyacrylic acid and thiolated chitosan without a gold layer in between. For both types of particles the relaxivity ratio was at least 2-fold higher than that of the likely best performing commercial product Resovist®. In addition, cellular internalization was strongly improved along with lowered cytotoxicity. Because of these properties thiomer-coated iron oxide nanoparticles seem to be a promising tool for cell tracking [382–384].

Another interesting application of thiomers is in the field of biosensors. Soto et al., for instance, developed biocompatible biosensors using a thiomer linked NO coating material for glucose detection [385]. After implantation into Yorkshire-type piglets sensitivity of glucose sensors in both, short and long-time examinations, was improved showing a higher analytical performance with a detection limit of 1-21 mM glucose [386]. In another study a thiolated chitosan was designed as highly sensitive and selective sensor for Hg^{2+} exhibiting a detection limit of just 0.5 ppb [37].

5.4. Regenerative medicine

5.4.1. Tissue engineering

Apart from proteins such as collagen and gelatin that can be considered as 'natural thiolated polymers', thiomers have been introduced as new type of scaffolds for tissue engineering at the 4th Central European Symposium on Pharmaceutical Technology in Vienna in 2001 [398,399]. Since then they inspired scientists to develop more efficient biocompatible three-dimensional hydrogel matrices playing a key role as scaffolds for tissue engineering applications [400].

To facilitate, support or accelerate production of extracellular matrix (ECM) thiolated polymers are used as artificial ECM. Most established thiomer hydrogels consist of thiolated hyaluronan [401], thiolated gelatin [275], thiolated heparin [134], thiolated collagen [208] and thiolated chitosan [402] [403]. The type of thiomer and degree of functionalization strongly affects the properties of the hydrogel such as swelling and degradation behavior as explained in detail in chapter 2. Moreover, the versatility of porosity, mesh size, surface area and density adjusted by the degree of crosslinking affect cell immigration [403,404]. In addition, the type of crosslinking reaction affects strength and linking time of hydrogels as well [405]. Thiomer hydrogels facilitate ECM production with widespread glycosaminoglycan matrix deposition equal to cell-mediated degradation of biosynthetic scaffolds [406,407].

Furthermore, drug release controlling properties of thiomers have been discovered for tissue engineering. Vogt et al., for instance, developed a light triggered NO releasing nanofibrous matrix using thiolated gelatin. As NO release was accurately controlled showing antimicrobial activity against S. aureus, this thiomer may be beneficial to replace conventional gelatin in tissue engineering [408]. Grim et al. established a thiomer ECM possessing capability of repeatable signal protein delivery releasing proteins on demand. Repeatability is achieved by the photoinduced release of protein recovering the radical functionality and gaining thereby possibility to 'click' other proteins [409].

Another advantage of thiomers in tissue engineering is the possibility to print scaffolds with them [209]. Bioprintable scaffolds with sufficient cell viability and proliferation were implemented by thiomer coated porous dextran beads and gold particles establishing the option to remove the artificial scaffold by dissolution with N-acetyl-L-cysteine [410–412]. Crosslinking with a 4-arm tetra acrylate led to higher stiffness of gel improving printability without diminish cell growth and proliferation [96]. Further improvements made up to 20 layers of printed 3D network possible with capability to encapsulate viable stem cells [413]. Recently, a printable scaffold was generated without taking toxic risks of photoinitiators and additionally possessing target specific functionalization of hydrogels by copper-catalyzed azide-alkyne cycloaddition chemistry after printing [414].

5.4.2. Tissue regeneration

The great and versatile potential of thiolated polymers in the field of tissue regeneration has been demonstrated in numerous studies. Within this review we highlight just a view of them providing an overview about the benefits and different research directions having arisen within recent years.

In particular in the field of anatomic tissue regeneration thiomers seem to be advantageous over other polymers. Autologous bone marrow-derived mesenchymal stromal cells, for instance, were encapsulated in a scaffold of thiolated hyaluronan and thiolated gelatin. Implantation in rabbits with defects in the patellar groove of femoral articular cartilage facilitated osteogenesis and chondrogenesis leading to repair of cartilage and trabecular bone within three months [415]. This great potential of thiolated hyaluronan and thiolated gelatin for the encapsulation of mesenchymal stromal cells was also shown in numerous other studies e.g. [416,417]. Furthermore, human osteoarthritic chondrocytes were encapsulated in a thiolated hyaluronic acid hydrogel showing already after two weeks increased production of cartilage-specific components such as collagen and sulfated glycosaminoglycans [391,418]. Thiomer ECMs were also shown to repair body organs. For trachea regeneration, for instance, human tracheal scar fibroblasts were successfully encapsulated in a thiolated hyaluronan retaining their phenotype and exhibiting secretion of ECM *in vivo* [419]. In addition, thiomers have already shown potential to treat neural diseases. Encapsulation of chick dorsal root ganglia resulted in > 50% increase in neurite length of cultures in comparison with fibrin hydrogel [420]. Even in treatment of stroke thiomers seem to be advantageous. Especially for administration into the stroke area, hydrogels based on thiolated hyaluronic acid create a favorable environment for reasons such as the same viscoelastic properties as the brain or inhibition of scar formation and of immunological responses [421,422]. Moreover, thiolated polymers have also shown potential for myocardial tissue regeneration. Intramyocardial injection of human cardiosphere-derived cells (CDCs) embedded in a crosslinked hyaluronan matrix evoked an amplification in left ventricular ejection fraction as well as cardiovascular differentiation of encapsulated CDCs after creating myocardial infarction in mice [423].

Another research direction is focusing on the use of thiomers in cancer treatment. As the number of cancer diseases is increasing, the discovery of novel more potent therapies is urgently needed. Thiomer hydrogels containing appropriate cells might be a promising strategy. Scaffolds containing thiomers successfully encapsulated stem cells with respect to spreading, migration and proliferation *in vivo* [424–426]. Encapsulated stem cells offered possibility in cancer treatment by creation of a secondary tumor causing an endogenous immune response. Subcutaneously implantation in nude mice distant from primary tumor indicated a potent antitumor response and tumor regression [427].

5.5. Cosmetics

5.5.1. Antiperspirants

Both for treatment of hyperhidrosis and for antiperspirants aluminium salts such as $AlCl_3$ play a central role [428]. Aluminium salts, however, are getting increasingly under suspect to provoke diseases such as Alzheimer disease or cancer [429,430]. Meanwhile, consumers prefer therefore aluminium-free products. Accordingly, the development of aluminium-free antiperspirants is of great commercial interest. A promising alternative to aluminium salts are thiomers, as they crosslink with the mucus of sweat glands via disulfide bond formation instead of just ionically crosslinking as aluminium does leading to a mechanical obstruction of the ducts [431]. For this purpose thiolated polyacrylates and thiolated polyethyleneimines were already tested [26]. Full potential of thiomers as antiperspirants, however, has so far not been reached. Thiomers can be oxidized on the skin before they reach their target in the sweat glands. Furthermore, if they are too big in size, they likely do not get access to deeper regions of the ducts. As meanwhile on the one hand S-protected thiomers have been developed being stable towards oxidation interacting comparatively more efficiently with mucus [24,105], and on the other hand comparatively small thiomers such as star shaped thiolated PEGs [34] or thiolated cyclodextrins [178] are available, the potential for further improvements is obvious. In particular thiolated cyclodextrins would offer the additional advantage of binding malodours causing molecules [432]. Furthermore, antimicrobials could be incorporated in thiolated cyclodextrins [433].

5.5.2. Hair treatments

The replacement of lost keratin seems to be a promising strategy in order to restore damaged hair structure. As native keratin is highly crosslinked via numerous disulfide bonds exhibiting just a few free thiol moieties, it can bind only to a minor extent to hair. Recently, however, keratin derivatives displaying highly reactive S-protected mercapto substructures were generated. These derivatives showed a strongly enhanced permanent binding to hair fibres (Fig. 10). Raising the content of free thiols in keratin and transforming these thiol substructures in disulfides with mercaptonicotinic acid seems therefore to be a promising concept for a durable binding of keratin to hair [210]. Furthermore, dimethicone/mercapto propyl methicone copolymers as illustrated in Fig. 6 were shown to remain associated with the hair cuticle up to 100% even after 40 shampoo/comb/blow dry cycles. This strong binding of such thiomers to hair are in particular useful to extend the colour retention of colour-treated hair [77].

6. Conclusion

Evolution has produced thiol groups as the most important bridging structure because of the easy interconversion between thiols and disulfides and as essential functional group facilitating numerous physiological processes. Endogenous peptides and proteins would tremendously lose functionality without cysteine - the likely most intriguing and diverse amino acid of all twenty - and life might simply not exist at all without it. Transferring this crucial biological role of thiols to synthetic polymers opens up huge possibilities. ***The great potential of thiolation of synthetic polymers might be described in the best way by the simple imagination of protein chemistry with and without cysteine.*** Although the progress and achievements made since we have entered this new era of polymer chemistry twenty years ago are impressive, we have just taken a few steps on a long, long way to go. These few steps include overall knowledge gained in the field of drug delivery and regenerative medicine and can be summarized as following. Due to the covalent attachment of thiol groups to numerous natural and synthetic polymers high adhesive properties through disulfide bond formation in particular to cysteine-rich proteins such as mucus glycoproteins, keratins and certain cell membrane-associated proteins are provided (I), high *in situ* gelling properties are gained through disulfide crosslinking within the thiolated polymer per se (II), and by interacting with thiol substructures of endogenous functional proteins, effects such as enzyme inhibition, permeation enhancement or efflux pump inhibition can be achieved (III). These unique properties make thiomers to a versatile tool for new drugs, drug delivery systems, diagnostics, regenerative medicine, cosmetics and even textiles.

The greatest challenges in front of us are certainly the deepening and improvement of our knowledge about physiological processes in that thiols are involved and the design of thiomers participating in such physiological processes in a way that highly beneficial therapeutic effects can be achieved. The enormous potential of this strategy has already been shown by various developments where thiomers were employed to mimic natural mechanisms and to interact with biomaterials via disulfide bond formation. However, the authors are convinced that these so far established systems are just the tip of the iceberg because endogenous thiols are involved in almost all physiological processes providing a sheer inexhaustible source for sound further applications. The door is open and will reward in particular all those that are experts on the interphase between life sciences and polymer chemistry being willing to enter and to break new ground in this booming field.

References

[1] A. Bernkop-Schnürch, Process for Improving the Mucoadhesion of Polymers, and the Preparation and use of these, in: AT406054 (B), 1998.
[2] A. Bernkop-Schnürch, V. Schwarz, S. Steininger, Polymers with thiol groups: a new generation of mucoadhesive polymers? Pharm. Res. 16 (1999) 876–881.
[3] A. Bernkop-Schnürch, Thiomers: a new generation of mucoadhesive polymers, Adv. Drug Deliv. Rev. 57 (2005) 1569–1582.
[4] A. Bernkop-Schnürch, S. Steininger, Synthesis and characterisation of mucoadhesive thiolated polymers, Int. J. Pharm. 194 (2000) 239–247.
[5] Y. Dong, A.O. Saeed, W. Hassan, C. Keigher, Y. Zheng, H. Tai, A. Pandit, W. Wang, "One-step" preparation of thiol-ene clickable PEG-based thermoresponsive hyperbranched copolymer for *in situ* crosslinking hybrid hydrogel, Macromol. Rapid Commun. 33 (2012) 120–126.
[6] Y. Fu, K. Xu, X. Zheng, A.J. Giacomin, A.W. Mix, W.J. Kao, 3D cell entrapment in crosslinked thiolated gelatin-poly(ethylene glycol) diacrylate hydrogels, Biomaterials 33 (2012) 48–58.
[7] F. Hintzen, F. Laffleur, F. Sarti, G. Shahnaz, A. Bernkop-Schnürch, Thiomers: influence of molar mass on *in situ* gelling properties, Int. J. Pharm. 436 (2012) 120–126.
[8] A. Bernkop-Schnürch, A.E. Clausen, M. Hnatyszyn, Thiolated polymers: synthesis and *in vitro* evaluation of polymer–cysteamine conjugates, Int. J. Pharm. 226 (2001) 185–194.
[9] G.H. Snyder, M.K. Reddy, M.J. Cennerazzo, D. Field, Use of local electrostatic environments of cysteines to enhance formation of a desired species in a reversible disulfide exchange reaction, Biochim. Biophys. Acta 749 (1983) 219–226.
[10] T.F. Palmberger, K. Albrecht, B. Loretz, A. Bernkop-Schnürch, Thiolated polymers: evaluation of the influence of the amount of covalently attached L-cysteine to poly(acrylic acid), Eur. J. Pharm. Biopharm. 66 (2007) 405–412.
[11] C. Menzel, J. Silbernagl, F. Laffleur, C. Leichner, M. Jelkmann, C.W. Huck, S. Hussain, A. Bernkop-Schnürch, 2,2′Dithiodinicotinyl ligands: Key to more reactive thiomers, Int. J. Pharm. 503 (2016) 199–206.
[12] J.M. Wilson, R.J. Bayer, D.J. Hupe, Structure-reactivity correlations for the thiol-disulfide interchange reaction, J. Am. Chem. Soc. 99 (1977) 7922–7926.
[13] P. Nagy, Kinetics and mechanisms of thiol-disulfide exchange covering direct substitution and thiol oxidation-mediated pathways, Antioxid. Redox Signal. 18 (2013) 1623–1641.
[14] G. Millotti, F. Laffleur, G. Perera, C. Vigl, K. Pickl, F. Sinner, A. Bernkop-Schnürch, *In vivo* evaluation of thiolated chitosan tablets for oral insulin delivery, J. Pharm. Sci. 103 (2014) 3165–3170.
[15] S.G. Tajc, B.S. Tolbert, R. Basavappa, B.L. Miller, Direct determination of thiol pKa by isothermal titration microcalorimetry, J. Am. Chem. Soc. 126 (2004) 10508–10509.
[16] G.H. Snyder, M.J. Cennerazzo, A.J. Karalis, D. Field, Electrostatic influence of local cysteine environments on disulfide exchange kinetics, Biochemistry 20 (1981) 6509–6519.
[17] L.B. Poole, The basics of thiols and cysteines in redox biology and chemistry, Free Radic. Biol. Med. 80 (2015) 148–157.
[18] A. Allen, D. Hutton, S. McQueen, A. Garner, Dimensions of gastroduodenal surface pH gradients exceed those of adherent mucus gel layers, Gastroenterology 85 (1983) 463–466.
[19] C. Lehr, F. Poelma, H. Junginger, J. Tukker, An estimate of turnover time of intestinal mucus gel layer in the rat *in situ* loop, Int. J. Pharm. 70 (1991) 235–240.
[20] A. Bernkop-Schnürch, M. Hornof, T. Zoidl, Thiolated polymers–thiomers: synthesis and *in vitro* evaluation of chitosan-2-iminothiolane conjugates, Int. J. Pharm. 260 (2003) 229–237.

[21] V.M. Leitner, G.F. Walker, A. Bernkop-Schnürch, Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins, Eur. J. Pharm. Biopharm. 56 (2003) 207–214.

[22] M. Budai-Szűcs, G. Horvát, B. Gyarmati, B. Szilágyi, A. Szilágyi, S. Berkó, R. Ambrus, P. Szabó-Révész, G. Sandri, M.C. Bonferoni, C. Caramella, E. Csányi, The effect of the antioxidant on the properties of thiolated poly(aspartic acid) polymers in aqueous ocular formulations, Eur. J. Pharm. Biopharm. 113 (2017) 178–187.

[23] S. Dünnhaupt, J. Barthelmes, D. Rahmat, K. Leithner, C.C. Thurner, H. Friedl, A. Bernkop-Schnürch, S-protected thiolated chitosan for oral delivery of hydrophilic macromolecules: evaluation of permeation enhancing and efflux pump inhibitory properties, Mol. Pharm. 9 (2012) 1331–1341.

[24] J. Iqbal, G. Shahnaz, S. Dünnhaupt, C. Müller, F. Hintzen, A. Bernkop-Schnürch, Preactivated thiomers as mucoadhesive polymers for drug delivery, Biomaterials 33 (2012) 1528–1535.

[25] S. Bonengel, A. Bernkop-Schnürch, Thiomers–from bench to market, J. Control. Release 195 (2014) 120–129.

[26] J.P. Courtois, W. Liu, I.K. Smith, L. Wang, M.S. White, Q. Zhang, Anitperspirant Compositions, W. (A1) (Ed.) https://worldwide.espacenet.com/publicationDetails/biblio?FT=D&date=20070628&DB=EPODOC&locale=en_EP&CC=WO&NR=2007071375A1&KC=A1&ND=5#2007.

[27] K. Kafedjiiski, M. Hoffer, M. Werle, A. Bernkop-Schnürch, Improved synthesis and *in vitro* characterization of chitosan-thioethylamidine conjugate, Biomaterials 27 (2006) 127–135.

[28] E. Verheyen, S. van der Wal, H. Deschout, K. Braeckmans, S. de Smedt, A. Barendregt, W.E. Hennink, C.F. van Nostrum, Protein macromonomers containing reduction-sensitive linkers for covalent immobilization and glutathione triggered release from dextran hydrogels, J. Control. Release 156 (2011) 329–336.

[29] Y. Zambito, S. Fogli, C. Zaino, F. Stefanelli, M.C. Breschi, G. Di Colo, Synthesis, characterization and evaluation of thiolated quaternary ammonium-chitosan conjugates for enhanced intestinal drug permeation, Eur. J. Pharm. Sci. 38 (2009) 112–120.

[30] L. Maleki, U. Edlund, A.C. Albertsson, Thiolated hemicellulose as a versatile platform for one-pot click-type hydrogel synthesis, Biomacromolecules 16 (2015) 667–674.

[31] K.B. Miles, R.L. Ball, H.W. Matthew, Chitosan films with improved tensile strength and toughness from N-acetyl-cysteine mediated disulfide bonds, Carbohydr. Polym. 139 (2016) 1–9.

[32] A. Dubbini, R. Censi, M.E. Butini, M.G. Sabbieti, D. Agas, T. Vermonden, P. Di Martino, Injectable hyaluronic acid/PEG-p(HPMAm-lac)-based hydrogels dually cross-linked by thermal gelling and Michael addition, Eur. Polym. J. 72 (2015) 423–437.

[33] R.M. Dicharry, P. Ye, G. Saha, E. Waxman, A.D. Asandei, R.S. Parnas, Wheat gluten-thiolated poly(vinyl alcohol) blends with improved mechanical properties, Biomacromolecules 7 (2006) 2837–2844.

[34] O.C.J. Andren, M. Malkoch, Facile thiolation of hydroxyl functional polymers, Polym. Chem. 8 (2017) 4996–5001.

[35] D. Rahmat, C. Müller, J. Barthelmes, G. Shahnaz, R. Martien, A. Bernkop-Schnürch, Thiolated hydroxyethyl cellulose: design and *in vitro* evaluation of mucoadhesive and permeation enhancing nanoparticles, Eur. J. Pharm. Biopharm. 83 (2013) 149–155.

[36] M.T. Rojas, R. Königer, F.J. Stoddart, A.E. Kaifer, Supported monolayers containing preformed binding sites. Synthesis and interfacial binding properties of a thiolated ß-cyclodextrin derivative, J. Am. Chem. Soc. 117 (1995) 336–343.

[37] K. Chauhan, P. Singh, R.K. Singhal, New chitosan-thiomer: an efficient colorimetric sensor and effective sorbent for mercury at ultralow concentration, ACS Appl. Mater. Interfaces 7 (2015) 26069–26078.

[38] L. Solhi, S.A. Schönbichler, S. Dünnhaupt, J. Barthelmes, H. Friedl, C.W. Huck, A. Bernkop-Schnürch, Synthesis and *in vitro* characterization of a preactivated thiomer via polymerization reaction, Biomacromolecules 13 (2012) 3054–3063.

[39] J.H. Al Mahrooqi, E.A. Mun, A.C. Williams, V.V. Khutoryanskiy, Controlling the size of thiolated organosilica nanoparticles, Langmuir 34 (2018) 8347–8354.

[40] N.V. Tsarevsky, K. Matyjaszewski, Combining atom transfer radical polymerization and disulfide/thiol redox chemistry: A route to well-defined (bio)degradable polymeric materials, Macromolecules 38 (2005) 3087–3092.

[41] K. Kafedjiiski, A.H. Krauland, M.H. Hoffer, A. Bernkop-Schnürch, Synthesis and *in vitro* evaluation of a novel thiolated chitosan, Biomaterials 26 (2005) 819–826.

[42] M. Bhatia, M. Ahuja, H. Mehta, Thiol derivatization of Xanthan gum and its evaluation as a mucoadhesive polymer, Carbohydr. Polym. 131 (2015) 119–124.

[43] N. Mahanta, S. Valiyaveettil, Surface modified electrospun poly(vinyl alcohol) membranes for extracting nanoparticles from water, Nanoscale 3 (2011) 4625–4631.

[44] X. Zhu, M. Su, S. Tang, L. Wang, X. Liang, F. Meng, Y. Hong, Z. Xu, Synthesis of thiolated chitosan and preparation nanoparticles with sodium alginate for ocular drug delivery, Mol. Vis. 18 (2012) 1973–1982.

[45] R.A. Baus, J. Innerhofer, J. Rohrer, N. Lupo, A. Bernkop-Schnürch, Anhydrous thiomers: strategy for enhanced mucoadhesion, Eur. J. Pharm. Biopharm. 129 (2018) 273–281.

[46] A. Bernkop-Schnürch, M. Hornof, D. Guggi, Thiolated chitosans, Eur. J. Pharm. Biopharm. 57 (2004) 9–17.

[47] D. Liu, J. Li, H. Pan, F. He, Z. Liu, Q. Wu, C. Bai, S. Yu, X. Yang, Potential advantages of a novel chitosan-N-acetylcysteine surface modified nanostructured lipid carrier on the performance of ophthalmic delivery of curcumin, Sci. Rep. 6 (2016), 28796.

[48] J. Groll, S. Singh, K. Albrecht, M. Moeller, Biocompatible and degradable nanogels via oxidation reactions of synthetic thiomers in inverse miniemulsion, J. Polym. Sci. Part A: Polym. Chem. 47 (2009) 5543–5549.

[49] F. Hintzen, S. Hauptstein, G. Perera, A. Bernkop-Schnürch, Synthesis and *in vitro* characterization of entirely S-protected thiolated pectin for drug delivery, Eur. J. Pharm. Biopharm. 85 (2013) 1266–1273.

[50] M. Susa, A.K. Iyer, K. Ryu, F.J. Hornicek, H. Mankin, M.M. Amiji, Z. Duan, Doxorubicin loaded polymeric nanoparticulate delivery system to overcome drug resistance in osteosarcoma, BMC Cancer 9 (2009) 399.

[51] M. Ijaz, M. Prantl, N. Lupo, F. Laffleur, M. Hussain Asim, B. Matuszczak, A. Bernkop-Schnürch, Development of pre-activated α-cyclodextrin as a mucoadhesive excipient for intra-vesical drug delivery, Int. J. Pharm. 534 (2017) 339–347.

[52] F. Sarti, A. Staaf, D. Sakloetsakun, A. Bernkop-Schnürch, Thiolated hydroxyethylcellulose: synthesis and *in vitro* evaluation, Eur. J. Pharm. Biopharm. 76 (2010) 421–427.

[53] W. Suchaoin, I. Pereira de Sousa, K. Netsomboon, J. Rohrer, P. Hoffmann Abad, F. Laffleur, B. Matuszczak, A. Bernkop-Schnürch, Mucoadhesive polymers: synthesis and *in vitro* characterization of thiolated poly(vinyl alcohol), Int. J. Pharm. 503 (2016) 141–149.

[54] A. Jalil, B. Matuszczak, N.-M. Nguyen Le, A. Mahmood, F. Laffleur, A. Bernkop-Schnürch, Synthesis and characterization of thiolated PVP–Iodine complexes: key to highly mucoadhesive antimicrobial gels, Mol. Pharm. 15 (2018) 3527–3534.

[55] K.E. Swindle-Reilly, M. Shah, P.D. Hamilton, T.A. Eskin, S. Kaushal, N. Ravi, Rabbit study of an *in situ* forming hydrogel vitreous substitute, Invest. Ophthalmol. Vis. Sci. 50 (2009) 4840–4846.

[56] S. Dünnhaupt, J. Barthelmes, S. Köllner, D. Sakloetsakun, G. Shahnaz, A. Düregger, A. Bernkop-Schnürch, Thiolated nanocarriers for oral delivery of hydrophilic macromolecular drugs, Carbohydr. Polym. 117 (2015) 577–584.

[57] P. Esmaeilzadeh, M. Menzel, T. Groth, Cyclic redox-mediated switching of surface properties of thiolated polysaccharide multilayers and its effect on fibroblast adhesion, ACS Appl. Mater. Interfaces 10 (2018) 31168–31177.

[58] M. Liras, O. Garcia, N. Guarrotxena, M. Palacios-Cuesta, I. Quijada-Garrido, Versatile thiolated thermosensitive polymers synthesized by ATRP of MEO_2MA and AcSEMA, a new methacrylic monomer with a protected thiol group, Polym. Chem. 4 (2013) 5751.

[59] M.T. Cook, S.A. Schmidt, E. Lee, W. Samprasit, P. Opanasopit, V.V. Khutoryanskiy, Synthesis of mucoadhesive thiol-bearing microgels from 2-(acetylthio)ethylacrylate and 2-hydroxyethylmethacrylate: novel drug delivery systems for chemotherapeutic agents to the bladder, J. Mater. Chem. B 3 (2015) 6599–6604.

[60] J. Kamada, K. Koynov, C. Corten, A. Juhari, J.A. Yoon, M.W. Urba, A.C. Balazs, K. Matyjaszewski, Redox responsive behavior of thiol/disulfide-functionalized star polymers synthesized via atom transfer radical polymerization, Macromolecules 43 (2010) 4133–4139.

[61] N. Murthy, J. Campbell, N. Fausto, A.S. Hoffman, P.S. Stayton, Bioinspired pH-responsive polymers for the intracellular delivery of biomolecular drugs, Bioconjug. Chem. 14 (2003) 412–419.

[62] I. Ullah, K. Muhammad, M. Akpanyung, A. Nejjari, A.L. Neve, J. Guo, Y. Feng, C. Shi, Bioreducible, hydrolytically degradable and targeting polymers for gene delivery, J. Mater. Chem. B 5 (2017) 3253–3276.

[63] T.-i. Kim, S.W. Kim, Bioreducible polymers for gene delivery, React. Funct. Polym. 71 (2011) 344–349.

[64] Z. Guo, H. Tian, J. Xia, J. Chen, L. Lin, X. Chen, Bioreducible crosslinked low molecular weight branched PEI-PBLG as an efficient gene carrier, Sci. China Chem. 53 (2010) 2490–2496.

[65] J. Liu, Y. Xu, Q. Yang, C. Li, W.E. Hennink, R. Zhuo, X. Jiang, Reduction biodegradable brushed PDMAEMA derivatives synthesized by atom transfer radical polymerization and click chemistry for gene delivery, Acta Biomater. 9 (2013) 7758–7766.

[66] D. Bontempo, K.L. Heredia, B.A. Fish, H.D. Maynard, Cysteine-reactive polymers synthesized by atom transfer radical polymerization for conjugation to proteins, J. Am. Chem. Soc. 126 (2004) 15372–15373.

[67] P.J. Roth, C. Boyer, A.B. Lowe, T.P. Davis, RAFT polymerization and thiol chemistry: a complementary pairing for implementing modern macromolecular design, Macromol. Rapid Commun. 32 (2011) 1123–1143.

[68] S. Ghosh, S. Basu, S. Thayumanavan, Simultaneous and reversible functionalization of copolymers for biological applications, Macromolecules 39 (2006) 5595–5597.

[69] L. Wong, C. Boyer, Z. Jia, H.M. Zareie, T.P. Davis, V. Bulmus, Synthesis of versatile thiol-reactive polymer scaffolds via RAFT polymerization, Biomacromolecules 9 (2008) 1934–1944.

[70] J.F. Quinn, M.R. Whittaker, T.P. Davis, Glutathione responsive polymers and their application in drug delivery systems, Polym. Chem. 8 (2017) 97–126.

[71] G. Moad, E. Rizzardo, S.H. Thang, Living radical polymerization by the RAFT process–a third update, Aust. J. Chem. 65 (2012) 985–1076.

[72] Y. Zhuang, Y. Su, Y. Peng, D. Wang, H. Deng, X. Xi, X. Zhu, Y. Lu, Facile fabrication of redox-responsive thiol-containing drug delivery system via RAFT polymerization, Biomacromolecules 15 (2014) 1408–1418.

[73] G.S. Irmukhametova, G.A. Mun, V.V. Khutoryanskiy, Thiolated mucoadhesive and PEGylated nonmucoadhesive organosilica nanoparticles from 3-mercaptopropyltrimethoxysilane, Langmuir 27 (2011) 9551–9556.

[74] S.J. Chiu, C.Y. Lin, H.C. Chou, T.M. Hu, Silica ouzo effect: amphiphilic drugs facilitate nanoprecipitation of polycondensed mercaptosilanes, Langmuir 32 (2016) 211–220.

[75] S.J. Chiu, S.Y. Wang, H.C. Chou, Y.L. Liu, T.M. Hu, Versatile synthesis of thiol- and amine-bifunctionalized silica nanoparticles based on the ouzo effect, Langmuir 30 (2014) 7676–7686.

[76] B.M. De, N. Daubresse, Composition pour la Coloration de Fibres Keratiniques Comprenant au Moins un Colorant Direct a Fonction Disulfure/thiol et au Moins un Polymere a Fonction thiol et Procede Utilisant la Composition, in L'Oreal SA, 2009.

[77] G. Hawkins, I.R. Afriat, J.H. Xavier, L.C. Popescu, Cosmetic compositions containing thiomers for hair color retention, in: S. United (Ed.), ELC Management LLC, 2011.

[78] A. Bernkop-Schnürch, M.D. Hornof, C.E. Kast, N. Langoth, Thiolated polymers: stability of thiol moieties under different storage conditions, Sci. Pharm. 70 (2002).

[79] J.L. Ruano García, A. Parra, J. Alemán, Efficient synthesis of disulfides by air oxidation of thiols under sonication, Green Chem. 10 (2008) 706–711.

[80] S. Singh, F. Topuz, K. Hahn, K. Albrecht, J. Groll, Embedding of active proteins and living cells in redox-sensitive hydrogels and nanogels through enzymatic cross-linking, Angew. Chem. Int. Ed. Eng. 52 (2013) 3000–3003.

[81] D. Sakloetsakun, J.M. Hombach, A. Bernkop-Schnürch, *In situ* gelling properties of chitosan-thioglycolic acid conjugate in the presence of oxidizing agents, Biomaterials 30 (2009) 6151–6157.

[82] B.D. Mather, K. Viswanathan, K.M. Miller, T.E. Long, Michael addition reactions in macromolecular design for emerging technologies, Prog. Polym. Sci. 31 (2006) 487–531.

[83] T.O. Machado, C. Sayer, P.H. Araujo, Thiol-ene polymerisation: a promising technique to obtain novel biomaterials, Eur. Polym. J. 86 (2017) 200–215.

[84] A.A. Aimetti, A.J. Machen, K.S. Anseth, Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme-responsive protein delivery, Biomaterials 30 (2009) 6048–6054.

[85] C.S. Ki, T.-Y. Lin, M. Korc, C.-C. Lin, Thiol-ene hydrogels as desmoplasia-mimetic matrices for modeling pancreatic cancer cell growth, invasion, and drug resistance, Biomaterials 35 (2014) 9668–9677.

[86] Y. Cao, J. He, J. Liu, M. Zhang, P. Ni, Folate-conjugated polyphosphoester with reversible cross-linkage and reduction sensitivity for drug delivery, ACS Appl. Mater. Interfaces 10 (2018) 7811–7820.

[87] M. Van Dijk, D.T. Rijkers, R.M. Liskamp, C.F. van Nostrum, W.E. Hennink, Synthesis and applications of biomedical and pharmaceutical polymers via click chemistry methodologies, Bioconjug. Chem. 20 (2009) 2001–2016.

[88] S.A. Robb, B.H. Lee, R. McLemore, B.L. Vernon, Simultaneously physically and chemically gelling polymer system utilizing a poly (NIPAAm-co-cysteamine)-based copolymer, Biomacromolecules 8 (2007) 2294–2300.

[89] J.-A. Yang, J. Yeom, B.W. Hwang, A.S. Hoffman, S.K. Hahn, *In situ*-forming injectable hydrogels for regenerative medicine, Prog. Polym. Sci. 39 (2014) 1973–1986.

[90] X.Z. Shu, Y. Liu, F. Palumbo, G.D. Prestwich, Disulfide-crosslinked hyaluronan-gelatin hydrogel films: a covalent mimic of the extracellular matrix for *in vitro* cell growth, Biomaterials 24 (2003) 3825–3834.

[91] K. Wang, J. Lu, R. Yin, L. Chen, S. Du, Y. Jiang, Q. Yu, Preparation and properties of cyclic acetal based biodegradable gel by thiol-ene photopolymerization, Mater. Sci. Eng. C 33 (2013) 1261–1266.

[92] J.C. Grim, I.A. Marozas, K.S. Anseth, Thiol-ene and photo-cleavage chemistry for controlled presentation of biomolecules in hydrogels, J. Control. Release 219 (2015) 95–106.

[93] C. Resetco, B. Hendriks, N. Badi, F. Du Prez, Thiol–ene chemistry for polymer coatings and surface modification – building in sustainability and performance, Mater. Horiz. 4 (2017) 1041–1053.

[94] M. Arslan, T.N. Gevrek, R. Sanyal, A. Sanyal, Fabrication of poly(ethylene glycol)-based cyclodextrin containing hydrogels via thiol-ene click reaction, Eur.Polymer J. 62 (2015).

[95] J. You, S.A. Park, D.S. Shin, D. Patel, V.K. Raghunathan, M. Kim, C.J. Murphy, G. Tae, A. Revzin, Characterizing the effects of heparin gel stiffness on function of primary hepatocytes, Tissue Eng. Part A 19 (2013) 2655–2663.

[96] A. Skardal, J. Zhang, G.D. Prestwich, Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates, Biomaterials 31 (2010) 6173–6181.

[97] S. Stichler, T. Jungst, M. Schamel, I. Zilkowski, M. Kuhlmann, T. Böck, T. Blunk, J. Teßmar, J. Groll, Thiol-ene clickable poly(glycidol) hydrogels for biofabrication, Ann. Biomed. Eng. 45 (2017) 273–285.

[98] Q. Xu, S.A.Y. Gao, L. Guo, J. Creagh-Flynn, D. Zhou, U. Greiser, Y. Dong, F. Wang, H. Tai, W. Liu, W. Wang, A hybrid injectable hydrogel from hyperbranched PEG macromer as a stem cell delivery and retention platform for diabetic wound healing, Acta Biomater. 75 (2018) 63–74.

[99] J.D. Smart, The basics and underlying mechanisms of mucoadhesion, Adv. Drug Deliv. Rev. 57 (2005) 1556–1568.

[100] M. Roldo, M. Hornof, P. Caliceti, A. Bernkop-Schnürch, Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and *in vitro* evaluation, Eur. J. Pharm. Biopharm. 57 (2004) 115–121.

[101] D. Dangl, M. Hornof, M. Hoffer, C. Kuntner, T. Wanek, H. Kvaternik, *In vivo* evaluation of ocular residence time of ^{124}I-labelled thiolated chitosan in rabbits using MicroPET technology, ARVO Annu. Meet. Abstr. (2009).

[102] Evaluation of the Corneal Residence Time of Chitosan-N-acetylcysteine Eye Drops in Patients With Dry Eye Syndrome After Single and Multiple Instillation, , in https://clinicaltrials.gov/ct2/show/NCT01753752?term=chitosan&draw=4&rank=26.

[103] J. Griesser, G. Hetényi, A. Bernkop-Schnürch, Thiolated Hyaluronic Acid as versatile mucoadhesive polymer, Polymers 10 (2018) 243.

[104] F. Laffleur, A. Bernkop-Schnürch, Thiomers: promising platform for macromolecular drug delivery, Future Med. Chem. 4 (2012) 2205–2216.

[105] M. Ijaz, A. Bernkop-Schnürch, Preactivated thiomers: their role in drug delivery, Expert. Opin. Drug Deliv. 12 (2015) 1269–1281.

[106] K.U. Shah, S.U. Shah, N. Dilawar, G.M. Khan, S. Gibaud, Thiomers and their potential applications in drug delivery, Expert. Opin. Drug Deliv. 14 (2017) 601–610.

[107] S. Duggan, W. Cummins, O. O' Donovan, H. Hughes, E. Owens, Thiolated polymers as mucoadhesive drug delivery systems, Eur. J. Pharm. Sci. 100 (2017) 64–78.

[108] H. Tian, Z. He, C. Sun, C. Yang, P. Zhao, L. Liu, K.W. Leong, H.Q. Mao, Z. Liu, Y. Chen, Uniform core-shell nanoparticles with thiolated hyaluronic acid coating to enhance oral delivery of insulin, Adv. Healthc. Mater. 7 (2018), e1800285.

[109] I. Pereira de Sousa, W. Suchaoin, O. Zupančič, C. Leichner, A. Bernkop-Schnürch, Totally S-protected hyaluronic acid: evaluation of stability and mucoadhesive properties as liquid dosage form, Carbohydr. Polym. 152 (2016) 632–638.

[110] R.G. Mannino, A.N. Santiago-Miranda, P. Pradhan, Y. Qiu, J.C. Mejias, S.S. Neelapu, K. Roy, W.A. Lam, 3D microvascular model recapitulates the diffuse large B-cell lymphoma tumor microenvironment *in vitro*, Lab Chip 17 (2017) 407–414.

[111] G.L. Griffith, B. Wirostko, H.K. Lee, L.E. Cornell, J.S. McDaniel, D.O. Zamora, A.J. Johnson, Treatment of corneal chemical alkali burns with a crosslinked thiolated hyaluronic acid film, Burns 44 (2018) 1179–1186.

[112] D.L. Williams, B.K. Mann, A crosslinked HA-based hydrogel ameliorates dry eye symptoms in dogs, Int. J. Biomater. 2013 (2013), 460437.

[113] T.I. Zarembinski, N.J. Doty, I.E. Erickson, R. Srinivas, B.M. Wirostko, W.P. Tew, Thiolated hyaluronan-based hydrogels crosslinked using oxidized glutathione: an injectable matrix designed for ophthalmic applications, Acta Biomater. 10 (2014) 94–103.

[114] A. Bernkop-Schnürch, Y. Pinter, D. Guggi, H. Kahlbacher, G. Schöffmann, M. Schuh, I. Schmerold, M.D. Del Curto, M. D'Antonio, P. Esposito, C. Huck, The use of thiolated polymers as carrier matrix in oral peptide delivery–proof of concept, J. Control. Release 106 (2005) 26–33.

[115] F. Atyabi, F. Talaie, R. Dinarvand, Thiolated chitosan nanoparticles as an oral delivery system for Amikacin: *in vitro* and ex vivo evaluations, J. Nanosci. Nanotechnol. 9 (2009) 4593–4603.

[116] M. Werle, M. Hoffer, Glutathione and thiolated chitosan inhibit multidrug resistance P-glycoprotein activity in excised small intestine, J. Control. Release 111 (2006) 41–46.

[117] N. Denora, A. Lopedota, M. Perrone, V. Laquintana, R.M. Iacobazzi, A. Milella, E. Fanizza, N. Depalo, A. Cutrignelli, A. Lopalco, M. Franco, Spray-dried mucoadhesives for intravesical drug delivery using N-acetylcysteine- and glutathione-glycol chitosan conjugates, Acta Biomater. 43 (2016) 170–184.

[118] M. Perrone, A. Lopalco, A. Lopedota, A. Cutrignelli, V. Laquintana, M. Franco, A. Bernkop-Schnürch, N. Denora, S-preactivated thiolated glycol chitosan useful to combine mucoadhesion and drug delivery, Eur. J. Pharm. Biopharm. 132 (2018) 103–111.

[119] A. Makhlof, M. Werle, Y. Tozuka, H. Takeuchi, Nanoparticles of glycol chitosan and its thiolated derivative significantly improved the pulmonary delivery of calcitonin, Int. J. Pharm. 397 (2010) 92–95.

[120] L. Yin, J. Ding, C. He, L. Cui, C. Tang, C. Yin, Drug permeability and mucoadhesion properties of thiolated trimethyl chitosan nanoparticles in oral insulin delivery, Biomaterials 30 (2009) 5691–5700.

[121] X. Zhao, L. Yin, J. Ding, C. Tang, S. Gu, C. Yin, Y. Mao, Thiolated trimethyl chitosan nanocomplexes as gene carriers with high *in vitro* and *in vivo* transfection efficiency, J. Control. Release 144 (2010) 46–54.

[122] W. Cheng, C. Tang, C. Yin, Effects of particle size and binding affinity for small interfering RNA on the cellular processing, intestinal permeation and anti-inflammatory efficacy of polymeric nanoparticles, J. Gene Med. 17 (2015) 244–256.

[123] M. Rahbarian, E. Mortazavian, F.A. Dorkoosh, M. Rafiee Tehrani, Preparation, evaluation and optimization of nanoparticles composed of thiolated triethyl chitosan: a potential approach for buccal delivery of insulin, J. Drug Deliv. Sci. Tech. 44 (2018) 254–263.

[124] Y. Zambito, F. Felice, A. Fabiano, R. Di Stefano, G. Di Colo, Mucoadhesive nanoparticles made of thiolated quaternary chitosan crosslinked with hyaluronan, Carbohydr. Polym. 92 (2013) 33–39.

[125] A. Fabiano, A.M. Piras, G. Uccello-Barretta, F. Balzano, A. Cesari, L. Testai, V. Citi, Y. Zambito, Impact of mucoadhesive polymeric nanoparticulate systems on oral bioavailability of a macromolecular model drug, Eur. J. Pharm. Biopharm. 130 (2018) 281–289.

[126] A. Fabiano, P. Chetoni, Y. Zambito, Mucoadhesive nano-sized supramolecular assemblies for improved pre-corneal drug residence time, Drug Dev. Ind. Pharm. 41 (2015) 2069–2076.

[127] A.A. Kassem, R.M. Farid, D.A. Issa, D.S. Khalil, M.Y. Abd-El-Razzak, H.I. Saudi, H.M. Eltokhey, E.A. El-Zamarany, Development of mucoadhesive microbeads using thiolated sodium alginate for intrapocket delivery of resveratrol, Int. J. Pharm. 487 (2015) 305–313.

[128] A. Martínez, M. Benito-Miguel, I. Iglesias, J.M. Teijón, M.D. Blanco, Tamoxifen-loaded thiolated alginate-albumin nanoparticles as antitumoral drug delivery systems, J. Biomed. Mater. Res. Part A 100 (2012) 1467–1476.

[129] S.U. Shah, M. Socha, I. Fries, S. Gibaud, Synthesis of S-nitrosoglutathione-alginate for prolonged delivery of nitric oxide in intestines, Drug deliv. 23 (2016) 2927–2935.

[130] M. Davidovich-Pinhas, O. Harari, H. Bianco-Peled, Evaluating the mucoadhesive properties of drug delivery systems based on hydrated thiolated alginate, J. Control. Release 136 (2009) 38–44.

[131] G. Perera, J. Barthelmes, A. Bernkop-Schnürch, Novel pectin-4-aminothiophenole conjugate microparticles for colon-specific drug delivery, J. Control. Release 145 (2010) 240–246.

[132] A.L. Martins, A.C. de Oliveira, C.M. do Nascimento, L.A. Silva, M.P. Gaeti, E.M. Lima, S.F. Taveira, K.F. Fernandes, R.N. Marreto, Mucoadhesive properties of thiolated pectin-based pellets prepared by extrusion-spheronization technique, J. Pharm. Sci. 106 (2017) 1363–1370.

[133] K. Cheewatanakornkool, S. Niratisai, C.R. Dass, P. Sriamornsak, Redox-responsive microbeads containing thiolated pectin-doxorubicin conjugate inhibit tumor growth and metastasis: an *in vitro* and *in vivo* study, Int. J. Pharm. 545 (2018) 1–9.

[134] G. Tae, Y.J. Kim, W.I. Choi, M. Kim, P.S. Stayton, A.S. Hoffman, Formation of a novel heparin-based hydrogel in the presence of heparin-binding biomolecules, Biomacromolecules 8 (2007) 1979–1986.

[135] M. Devarasetty, E. Wang, S. Soker, A. Skardal, Mesenchymal stem cells support growth and organization of host-liver colorectal-tumor organoids and possibly resistance to chemotherapy, Biofabrication 9 (2017), 021002.

[136] E. Foster, J. You, C. Siltanen, D. Patel, A. Haque, L. Anderson, A. Revzin, Heparin hydrogel sandwich cultures of primary hepatocytes, Eur. Polym. J. 72 (2015) 726–735.

[137] P. Esmaeilzadeh, A. Köwitsch, F. Heyroth, G. Schmidt, S. Fischer, K. Richter, T. Groth, Synthesis of thiolated polysaccharides for formation of polyelectrolyte multilayers with improved cellular adhesion, Carbohydr. Polym. 157 (2017) 1205–1214.

[138] P. Esmaeilzadeh, A. Köwitsch, A. Liedmann, M. Menzel, B. Fuhrmann, G. Schmidt, J. Klehm, T. Groth, Stimuli-responsive multilayers based on thiolated polysaccharides that affect fibroblast cell adhesion, ACS Appl. Mater. Interfaces 10 (2018) 8507–8518.

[139] A.H. Krauland, V.M. Leitner, A. Bernkop-Schnürch, Improvement in the *in situ* gelling properties of deacetylated gellan gum by the immobilization of thiol groups, J. Pharm. Sci. 92 (2003) 1234–1241.

[140] H. Mahajan, H. Shaikh, S. Gattani, P. Nerkar, *In situ* gelling system based on thiolated gellan gum as new carrier for nasal administration of dimenhydrinate, Int. J. pharm. Sci. Nanotech 2 (2009) 544–550.

[141] A.H. Bacelar, J. Silva-Correia, J.M. Oliveira, R.L. Reis, Recent progress in gellan gum hydrogels provided by functionalization strategies, J. Mater. Chem. B 4 (2016) 6164–6174.

[142] M.P. More, M.S. Bhamare, C.J. Bhavsar, P.O. Patil, P.K. Deshmukh, Development of novel thiolated carboxymethyl-gellan gum as potential mucoadhesive polymer: application of DoE, Adv. Mater. Sci. 2 (2017) 1–9.

[143] W. Suchaoin, S. Bonengel, S. Hussain, C.W. Huck, B.N. Ma, A. Bernkop-Schnürch, Synthesis and *in vitro* evaluation of thiolated carrageenan, J. Pharm. Sci. 104 (2015) 2523–2530.

[144] M. Jelkmann, S. Bonengel, C. Menzel, S. Markovic, A. Bernkop-Schnürch, New perspectives of starch: synthesis and *in vitro* assessment of novel thiolated mucoadhesive derivatives, Int. J. Pharm. 546 (2018) 70–77.

[145] C. Saikia, M. Das, A. Ramteke, T. Maji, Controlled release of curcumin from thiolated starchcoated iron oxide magnetic nanoparticles: an *in vitro* evaluation, Int. J. Polym. Mater. Polym. Biomater. 66 (2017) 349–358.

[146] K. Chauhan, V. Priya, P. Singh, G.S. Chauhan, S. Kumari, R.K. Singhal, A green and highly efficient sulfur functionalization of starch, RSC Adv. 5 (2015) 51762–51772.

[147] G. Leonaviciute, W. Suchaoin, B. Matuszczak, H.T. Lam, A. Mahmood, A. Bernkop-Schnürch, Preactivated thiolated pullulan as a versatile excipient for mucosal drug targeting, Carbohydr. Polym. 151 (2016) 743–751.

[148] Priya S.S., Rekha M.R., Redox sensitive cationic pullulan for efficient gene transfection and drug retention in C6 glioma cells, Int. J. Pharm. 530 (2017) 401–414.

[149] S.S. Priya, M.R. Rekha, Disulphide cross linked pullulan based cationic polymer for improved gene delivery and efflux pump inhibition, Colloids Surf. B: Biointerfaces 146 (2016) 879–887.

[150] M. Perrone, A. Lopedota, E. Liberati, V. Russo, A. Cutrignelli, V. Laquintana, I.P. de Sousa, M. Franco, S. Tongiani, N. Denora, A. Bernkop-Schnürch, Natural dendrimers: Synthesis and *in vitro* characterization of glycogen-cysteamine conjugates, Eur. J. Pharm. Biopharm. 115 (2017) 168–176.

[151] M. Perrone, A. Lopalco, A. Lopedota, A. Cutrignelli, V. Laquintana, J. Douglas, M. Franco, E. Liberati, V. Russo, S. Tongiani, N. Denora, A. Bernkop-Schnürch, Preactivated thiolated glycogen as mucoadhesive polymer for drug delivery, Eur. J. Pharm. Biopharm. 119 (2017) 161–169.

[152] C. He, L. Yin, Y. Song, C. Tang, C. Yin, Optimization of multifunctional chitosan-siRNA nanoparticles for oral delivery applications, targeting TNF-α silencing in rats, Acta Biomater. 17 (2015) 98–106.

[153] M.R. Bhalekar, R.V. Bargaje, P.G. Upadhaya, A.R. Madgulkar, S.J. Kshirsagar, Formulation of mucoadhesive gastric retentive drug delivery using thiolated xyloglucan, Carbohydr. Polym. 136 (2016) 537–542.

[154] A.D. Kulkarni, A.A. Joshi, C.L. Patil, P.D. Amale, H.M. Patel, S.J. Surana, V.S. Belgamwar, K.S. Chaudhari, C.V. Pardeshi, Xyloglucan: a functional biomacromolecule for drug delivery applications, Int. J. Biol. Macromol. 104 (2017) 799–812.

[155] A.R. Madgulkar, M.R. Bhalekar, A.A. Kadam, Improvement of oral bioavailability of lopinavir without co-administration of ritonavir using microspheres of thiolated xyloglucan, AAPS PharmSciTech 19 (2018) 293–302.

[156] P. Tingaut, R. Hauert, T. Zimmermann, Highly efficient and straightforward functionalization of cellulose films with thiol-ene click chemistry, J. Mater. Chem. 21 (2011) 16066–16076.

[157] Z. Zhang, Y. Cao, L. Chen, Z. Huang, Preparation of thiol-functionalized cellulose and its application to the removal of Hg (II) from water environment, Cellul. Chem. Technol. 51 (2017) 559–567.

[158] B. Geng, H. Wang, S. Wu, J. Ru, C. Tong, Y. Chen, H. Liu, S. Wu, X. Liu, Surface-tailored nanocellulose aerogels with thiol-functional moieties for highly efficient and selective removal of Hg(II) ions from water, ACS Sustain. Chem. Eng. 5 (2017) 11715–11726.

[159] L.R. Arcot, M. Lundahl, O.J. Rojas, J. Laine, Asymmetric cellulose nanocrystals: thiolation of reducing end groups via NHS–EDC coupling, Cellulose 21 (2014) 4209–4218.

[160] F. Laffleur, L. Bacher, K. Netsomboon, Design, characterization and *in vitro* evaluation of a novel thiolated polymer: preactivated carboxymethyl cellulose, Ther. Deliv. 7 (2016) 7–14.

[161] F. Laffleur, A. Messirek, Development of mucoadhesive thio-carboxymethyl cellulose for application in buccal delivery of drugs, Ther. Deliv. 7 (2016) 63–71.

[162] J.A. Griessinger, S. Bonengel, A. Partenhauser, M. Ijaz, A. Bernkop-Schnürch, Thiolated polymers: evaluation of their potential as dermoadhesive excipients, Drug Dev. Ind. Pharm. 43 (2017) 204–212.

[163] G. Leonaviciute, S. Bonengel, A. Mahmood, M. Ahmad Idrees, A. Bernkop-Schnürch, S-protected thiolated hydroxyethyl cellulose (HEC): Novel mucoadhesive excipient with improved stability, Carbohydr. Polym. 144 (2016) 514–521.

[164] D. Rahmat, F.A. Rahman, L. Nurhidayati, D.R. Laksmitawati, Synthesis and characterization of hydroxypropyl cellulose-cysteamine conjugate as a novel cationic thiomer with lipophilic properties, Int. J. App. Pharm. 11 (2019) 222–226.

[165] R. Choi, J. Yang, J. Choi, E.K. Lim, E. Kim, J.S. Suh, Y.M. Huh, S. Haam, Thiolated dextran-coated gold nanorods for photothermal ablation of inflammatory macrophages, Langmuir 26 (2010) 17520–17527.

[166] F.S.M. Tekie, M. Soleimani, A. Zakerian, M. Dinarvand, M. Amini, R. Dinarvand, E. Arefian, F. Atyabi, Glutathione responsive chitosan-thiolated dextran conjugated miR-145 nanoparticles targeted with AS1411 aptamer for cancer treatment, Carbohydr. Polym. 201 (2018) 131–140.

[167] M. Kiani, F.S. Mirzazadeh Tekie, M. Dinarvand, M. Soleimani, R. Dinarvand, F. Atyabi, Thiolated carboxymethyl dextran as a nanocarrier for colon delivery of hSET1 antisense: *in vitro* stability and efficiency study, Mater. Sci. Eng. C Mater. Biol. Appl. 62 (2016) 771–778.

[168] G. Shahnaz, J. Iqbal, D. Rahmat, G. Perera, F. Laffleur, D. Rossi, A. Bernkop-Schnürch, Development and *in vivo* characterization of a novel peptide drug delivery system providing extended plasma half life, J. Control. Release 157 (2012) 375–382.

[169] M. Bhatia Rupa, Thiol functionalization of flaxseed mucilage: Preparation, characterization and evaluation as mucoadhesive polymer, Int. J. Biol. Macromol. 126 (2019) 101–106.

[170] M. Zaman, M. Hanif, K. Sultana, Atta-Ur-Rehman, Synthesis of thiolated arabinoxylan and its application as sustained release mucoadhesive film former, Biomed. Mater. 13 (2018) (025019).

[171] M. Bhatia, M. Ahuja, H. Mehta, Thiol derivatization of Xanthan gum and its evaluation as a mucoadhesive polymer, Carbohydr. Polym. 131 (2015) 119–124.

[172] F. Laffleur, M. Michalek, Modified xanthan gum for buccal delivery—a promising approach in treating sialorrhea, Int. J. Biol. Macromol. 102 (2017) 1250–1256.

[173] C. Menzel, M. Jelkmann, F. Laffleur, A. Bernkop-Schnürch, Nasal drug delivery: Design of a novel mucoadhesive and *in situ* gelling polymer, Int. J. Pharm. 517 (2017) 196–202.

[174] C.H. Chen, Y.S. Lin, S.J. Wu, F.L. Mi, Mutlifunctional nanoparticles prepared from arginine-modified chitosan and thiolated fucoidan for oral delivery of hydrophobic and hydrophilic drugs, Carbohydr. Polym. 193 (2018) 163–172.

[175] M. Ijaz, M. Ahmad, N. Akhtar, F. Laffleur, A. Bernkop-Schnürch, Thiolated α-Cyclodextrin: the invisible choice to prolong ocular Drug residence time, J. Pharm. Sci. 105 (2016) 2848–2854.

[176] M. Ijaz, B. Matuszczak, D. Rahmat, A. Mahmood, S. Bonengel, S. Hussain, C.W. Huck, A. Bernkop-Schnürch, Synthesis and characterization of thiolated β-cyclodextrin as a novel mucoadhesive excipient for intra-oral drug delivery, Carbohydr. Polym. 132 (2015) 187–195.

[177] C. Manta, G. Peralta-Altier, L. Gioia, M.F. Méndez, G. Seoane, K. Ovsejevi, Synthesis of a thiol-β-cyclodextrin, a potential agent for controlling enzymatic browning in fruits and vegetables, J. Agric. Food Chem. 61 (2013) 11603–11609.

[178] A. Moghadam, M. Ijaz, M.H. Asim, A. Mahmood, M. Jelkmann, B. Matuszczak, A. Bernkop-Schnürch, Non-ionic thiolated cyclodextrins - the next generation, Int. J. Nanomedicine 13 (2018) 4003–4013.

[179] J.G. Cheng, Y.M. Zhang, Y. Liu, Supramolecular assembly of thiolated cyclodextrin and ferrocene derivative for controlled drug delivery, Chemnanomat 4 (2018) 758–763.

[180] M.H. Asim, A. Moghadam, M. Ijaz, A. Mahmood, R.X. Götz, B. Matuszczak, A. Bernkop-Schnürch, S-protected thiolated cyclodextrins as mucoadhesive oligomers for drug delivery, J. Colloid Interface Sci. 531 (2018) 261–268.

[181] J. Liu, J. Alvarez, W. Ong, A.E. Kaifer, Network Aggregates Formed by C60 and Gold Nanoparticles Capped with γ-Cyclodextrin Hosts, Nano Lett. 1 (2001) 57–60.

[182] K. Netsomboon, F. Laffleur, A. Bernkop-Schnürch, P-glycoprotein inhibitors: synthesis and *in vitro* evaluation of a preactivated thiomer, Drug Dev. Ind. Pharm. 42 (2016) 668–675.

[183] S. Duggan, O. O'Donovan, E. Owens, E. Duggan, H. Hughes, W. Cummins, Comparison of the mucoadhesive properties of thiolated polyacrylic acid to thiolated polyallylamine, Int. J. Pharm. 498 (2016) 245–253.

[184] Y. Zhao, Y. Li, J. Ge, N. Li, L.B. Li, Pluronic-poly (acrylic acid)-cysteine/Pluronic L121 mixed micelles improve the oral bioavailability of paclitaxel, Drug Dev. Ind. Pharm. 40 (2014) 1483–1493.

[185] E. Cevher, D. Sensoy, M.A. Taha, A. Araman, Effect of thiolated polymers to textural and mucoadhesive properties of vaginal gel formulations prepared with polycarbophil and chitosan, AAPS PharmSciTech 9 (2008) 953–965.

[186] G. Perera, J. Barthelmes, A. Vetter, C. Krieg, C. Uhlschmied, G.K. Bonn, A. Bernkop-Schnürch, Thiolated polycarbophil/glutathione: defining its potential as a permeation enhancer for oral drug administration in comparison to sodium caprate, Drug Deliv. 18 (2011) 415–423.

[187] S. Bonengel, S. Hauptstein, G. Leonaviciute, J. Griessinger, A. Bernkop-Schnürch, Thiolated alkyl-modified carbomers: novel excipients for mucoadhesive emulsions, Eur. J. Pharm. Biopharm. 75 (2015) 123–130.

[188] S. Bonengel, S. Haupstein, G. Perera, A. Bernkop-Schnürch, Thiolated and S-protected hydrophobically modified cross-linked poly (acrylic acid)–a new

generation of multifunctional polymers, Eur. J. Pharm. Biopharm. 88 (2014) 390–396.

[189] Y. Zhang, X. Du, G. Li, C. Cai, J. Xu, X. Tang, Thiolated eudragit-based nanoparticles for oral insulin delivery: preparation, characterization, and evaluation using intestinal epithelial cells *in vitro*, Macromol. Biosci. 14 (2014) 842–852.

[190] W.J. Lee, S. Cha, M. Shin, M.A. Islam, C.S. Cho, H.S. Yoo, Induction of Th1 polarized immune responses by thiolated Eudragit-coated F4 and F18 fimbriae of enterotoxigenic Escherichia coli, Eur. J. Pharm. Biopharm. 79 (2011) 226–231.

[191] I.A. Elbahwy, N. Lupo, H.M. Ibrahim, H.R. Ismael, A.A. Kasem, C. Caliskan, B. Matuszczak, A. Bernkop-Schnürch, Mucoadhesive self-emulsifying delivery systems for ocular administration of econazole, Int. J. Pharm. 541 (2018) 72–80.

[192] G. Leonaviciute, N.T. Adamovic, H.T. Lam, J. Rohrer, A. Partenhauser, A. Bernkop-Schnürch, Self-emulsifying drug delivery systems (SEDDS): proof-of-concept how to make them mucoadhesive, Eur. J. Pharm. Biopharm. 112 (2017) 51–57.

[193] F. Prüfert, S. Bonengel, C. Menzel, A. Bernkop-Schnürch, Enhancing the efficiency of thiomers: utilizing a highly mucoadhesive polymer as backbone for thiolation and preactivation, Eur. J. Pharm. Sci. 96 (2017) 309–315.

[194] K.E. Swindle, P.D. Hamilton, N. Ravi, *In situ* formation of hydrogels as vitreous substitutes: Viscoelastic comparison to porcine vitreous, J. Biomed. Mater. Res. Part A 87 (2008) 656–665.

[195] J. Liang, J.J. Struckhoff, H. Du, P.D. Hamilton, N. Ravi, Synthesis and characterization of *in situ* forming anionic hydrogel as vitreous substitutes, J Biomed Mater Res B Appl Biomater 105 (2017) 977–988.

[196] A. Partenhauser, F. Laffleur, J. Rohrer, A. Bernkop-Schnürch, Thiolated silicone oil: synthesis, gelling and mucoadhesive properties, Acta Biomater. 16 (2015) 169–177.

[197] M. Ben Haddada, J. Blanchard, S. Casale, J.-M. Krafft, A. Vallée, C. Méthivier, S. Boujday, Optimizing the immobilization of gold nanoparticles on functionalized silicon surfaces: amine- vs thiol-terminated silane, Gold Bull. 46 (2013) 335–341.

[198] M.A. Cole, C.N. Bowman, Evaluation of thiol-ene click chemistry in functionalized polysiloxanes, J. Polym. Sci. A Polym. Chem. 51 (2013) 1749–1757.

[199] T.M.M. Ways, W.M. Lau, K.W. Ng, V.V. Khutoryanskiy, Synthesis of thiolated, PEGylated and POZylated silica nanoparticles and evaluation of their retention on rat intestinal mucosa *in vitro*, Eur. J. Pharm. Sci. 122 (2018) 230–238.

[200] Z. Shang, L. Zhang, X. Zhao, S. Liu, D. Li, Removal of Pb(II), Cd(II) and Hg(II) from aqueous solution by mercapto-modified coal gangue, J. Environ. Manag. 231 (2019) 391–396.

[201] A. Fürst, R. Baus, N. Lupo, A. Bernkop-Schnürch, Entirely S-protected thiolated silicone: A novel solid hydrophobic muco- and skin adhesive, J. Pharm. Sci. press. (2019) (ahead of print).

[202] X.-N. Zhang, V. Hollimon, D. Brodus, A Method for attaching thiol groups directly on a silicon (111) substrate, Acta Phys. -Chim. Sin. 32 (2016) 2364–2368.

[203] X. Zhang, P. Gao, V. Hollimon, D. Brodus, A. Johnson, H. Hu, Surface thiolation of silicon for antifouling application, Chem. Central J. 12 (2018) 10.

[204] K. Eriksson, L. Johansson, E. Göthelid, L. Nyholm, S. Oscarsson, Manufacturing of anisotropic particles by site specific oxidation of thiols, J. Mater. Chem. 22 (2012) 7681–7683.

[205] S. Duggan, O. O'Donovan, E. Owens, W. Cummins, H. Hughes, Synthesis of mucoadhesive thiolated gelatin using a two-step reaction process, Eur. J. Pharm. Biopharm. 91 (2015) 75–81.

[206] T.T. Hoang Thi, Y. Lee, S.B. Ryu, D.H. Nguyen, K.D. Park, Enhanced tissue adhesiveness of injectable gelatin hydrogels through dual catalytic activity of horseradish peroxidase, Biopolymers 109 (2018).

[207] G. Xu, X. Wang, C. Deng, X. Teng, E.J. Suuronen, Z. Shen, Z. Zhong, Injectable biodegradable hybrid hydrogels based on thiolated collagen and oligo(acryloyl carbonate)-poly(ethylene glycol)-oligo(acryloyl carbonate) copolymer for functional cardiac regeneration, Acta Biomater. 15 (2015) 55–64.

[208] K. Yamauchi, N. Takeuchi, A. Kurimoto, T. Tanabe, Films of collagen crosslinked by S-S bonds: preparation and characterization, Biomaterials 22 (2001) 855–863.

[209] R. Gaetani, D.A.M. Feyen, V. Verhage, R. Slaats, E. Messina, K.L. Christman, A. Giacomello, P.A.F.M. Doevendans, J.P.G. Sluijter, Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction, Biomaterials 61 (2015) 339–348.

[210] C. Leichner, C. Steinbring, R.A. Baus, D. Baecker, R. Gust, A. Bernkop-Schnürch, Reactive keratin derivatives: a promising strategy for covalent binding to hair, J. Colloid Interface Sci. 534 (2018) 533–541.

[211] Z. Cheng, X. Chen, D. Zhai, F. Gao, T. Guo, W. Li, S. Hao, J. Ji, B. Wang, Development of keratin nanoparticles for controlled gastric mucoadhesion and drug release, Journal of nanobiotechnology 16 (2018) 24.

[212] T. Luo, S. Hao, X. Chen, J. Wang, Q. Yang, Y. Wang, Y. Weng, H. Wei, J. Zhou, B. Wang, Development and assessment of kerateine nanoparticles for use as a hemostatic agent, Mater. Sci. Eng. C 63 (2016) 352–358.

[213] D. Juriga, K. Nagy, A. Jedlovszky-Hajdu, K. Perczel-Kovach, Y.M. Chen, G. Varga, M. Zrinyi, Biodegradation and osteosarcoma cell cultivation on poly(aspartic acid) based hydrogels, ACS Appl. Mater. Interfaces 8 (2016) 23463–23476.

[214] M. Budai-Szűcs, E.L. Kiss, B.A. Szilágyi, A. Szilágyi, B. Gyarmati, S. Berkó, A. Kovacs, G. Horvát, Z. Aigner, J. Soós, E. Csányi, Mucoadhesive cyclodextrin-modified thiolated poly(aspartic acid) as a potential ophthalmic drug delivery system, Polymers 10 (2018) 199.

[215] V.K. Yellepeddi, R. Mohammadpour, S.P. Kambhampati, C. Sayre, M.K. Mishra, R.M. Kannan, H. Ghandehari, Pediatric oral formulation of dendrimer-N-acetyl-l-cysteine conjugates for the treatment of neuroinflammation, Int. J. Pharm. 545 (2018) 113–116.

[216] Y. Liu, G.N. Chiu, Dual-functionalized PAMAM dendrimers with improved P-glycoprotein inhibition and tight junction modulating effect, Biomacromolecules 14 (2013) 4226–4235.

[217] B.S. Day, L.R. Fiegland, E.S. Vint, W. Shen, J.R. Morris, M.L. Norton, Thiolated dendrimers as multi-point binding headgroups for DNA immobilization on gold, Langmuir 27 (2011) 12434–12442.

[218] T. Wang, X. Zhang, Y. Pan, X. Miao, Z. Su, C. Wang, X. Li, Fabrication of doxorubicin functionalized gold nanorod probes for combined cancer imaging and drug delivery, Dalton Trans. 40 (2011) 9789–9794.

[219] H. Yu, Z.-g. Feng, A.-y. Zhang, L.-g. Sun, L. Qian, Synthesis and characterization of three-dimensional crosslinked networks based on self-assemly of α-cyclodextrins with thiolated 4-arm PEG using a three-step oxidation, Soft Matter 2 (2006) 343–349.

[220] N.C. Kalarickal, S. Rimmer, P. Sarker, J.-C. Leroux, Thiol-Functionalized Poly(ethylene glycol)-b-polyesters: synthesis and characterization, Macromolecules 40 (2007) 1874–1880.

[221] S. Matsumoto, R.J. Christie, N. Nishiyama, K. Miyata, A. Ishii, M. Oba, H. Koyama, Y. Yamasaki, K. Kataoka, Environment-responsive block copolymer micelles with a disulfide cross-linked core for enhanced siRNA delivery, Biomacromolecules 10 (2009) 119–127.

[222] B. Oh, C.H. Lee, Development of thiolated-graphene quantum dots for regulation of ROS in macrophages, Pharm. Res. 33 (2016) 2736–2747.

[223] I. Pereira de Sousa, K. Buttenhauser, W. Suchaoin, A. Partenhauser, M. Perrone, B. Matuszczak, A. Bernkop-Schnürch, Thiolated graphene oxide as promising mucoadhesive carrier for hydrophobic drugs, Int. J. Pharm. 509 (2016) 360–367.

[224] A. Mirzaie, M. Hasanzadeh, A. Jouyban, Cross-linked chitosan/thiolated graphene quantum dots as a biocompatible polysaccharide towards aptamer immobilization, Int. J. Biol. Macromol. 123 (2019) 1091–1105.

[225] T. Yin, J. Liu, Z. Zhao, L. Dong, H. Cai, L. Yin, J. Zhou, M. Huo, Smart nanoparticles with a detachable outer shell for maximized synergistic antitumor efficacy of therapeutics with varying physicochemical properties, J. Control. Release 243 (2016) 54–68.

[226] Y. Hu, R. Wang, S. Wang, L. Ding, J. Li, Y. Luo, X. Wang, M. Shen, X. Shi, Multifunctional Fe3O4@Au core/shell nanostars: a unique platform for multimode imaging and photothermal therapy of tumors, Sci. Rep. 6 (2016), 28325.

[227] M. Budai-Szűcs, G. Horvát, B. Szilágyi, B. Gyarmati, A. Szilágyi, S. Berkó, P. Szabó-Révész, G. Sandri, M.C. Bonferoni, C. Caramella, J. Soós, A. Facskó, E. Csányi, Cationic thiolated poly(aspartamide) polymer as a potential excipient for artificial tear formulations, J. Ophthalmol. 2016 (2016), 2647264.

[228] M. Barbarić, M. Kralj, M. Marjanović, I. Husnjak, K. Pavelić, J. Filipović-Grčić, D. Zorc, B. Zorc, Synthesis and *in vitro* antitumor effect of diclofenac and fenoprofen thiolated and nonthiolated polyaspartamide-drug conjugates, Eur. J. Med. Chem. 42 (2007) 20–29.

[229] G. Braithwaite, Y. Svirkin, Thiolated PEG-PVA Hydrogels, US9873769B2 2018.

[230] J.A. Stefater, T.P. Stryjewski, Methods and polymer compositions for treating retinal detachment and other ocular disorders, WO2018013819 (A1) 2018.

[231] D.A. Ossipov, S. Piskounova, J.n. Hilborn, Poly (vinyl alcohol) cross-linkers for *in vivo* injectable hydrogels, Macromolecules 41 (2008) 3971–3982.

[232] C.O. Ibie, C.J. Thompson, R.M. Knott, Synthesis, characterisation and *in vitro* evaluation of novel thiolated derivatives of polyallylamine and quaternised polyallylamine, Colloid Polym. Sci. 293 (2015) 1737–1748.

[233] S. Duggan, H. Hughes, E. Owens, E. Duggan, W. Cummins, O. O' Donovan, Synthesis and characterisation of mucoadhesive thiolated polyallylamine, Int. J. Pharm. 499 (2016) 368–375.

[234] C. Vigl, K. Leithner, K. Albrecht, A. Bernkop-Schnürch, The efflux pump inhibitory properties of (thiolated) polyallylamines, J. Drug Del. Sci. Tech. 19 (2009) 405–411.

[235] H. Pan, S. Low, N. Weerasuriya, B. Wang, Y.S. Shon, Morphological transformation of gold nanoparticles on graphene oxide: effects of capping ligands and surface interactions, Nano Converg. 6 (2019) 2.

[236] B.J. Mishra, A. Kaul, P. Trivedi, L-Cysteine conjugated poly L-lactide nanoparticles containing 5-fluorouracil: formulation, characterization, release and uptake by tissues *in vivo*, Drug Deliv. 22 (2015) 214–222.

[237] X. Duan, R.S. Lewis, Improved haemocompatibility of cysteine-modified polymers via endogenous nitric oxide, Biomaterials 23 (2002) 1197–1203.

[238] P.N. Coneski, M.H. Schoenfisch, Synthesis of nitric oxide-releasing polyurethanes with s-nitrosothiol-containing hard and soft segments, Polym. Chem. 2 (2011) 906–913.

[239] J. Barthelmes, S. Dünnhaupt, S. Unterhofer, G. Perera, W. Schlocker, A. Bernkop-Schnürch, Thiolated particles as effective intravesical drug delivery systems for treatment of bladder-related diseases, Nanomedicine (London) 8 (2013) 65–75.

[240] M. Werle, K. Hironaka, H. Takeuchi, H. Hoyer, Development and *in vitro* characterization of liposomes coated with thiolated poly(acrylic acid) for oral drug delivery, Drug Dev. Ind. Pharm. 35 (2009) 209–215.

[241] K. Gradauer, S. Dünnhaupt, C. Vonach, H. Szöllösi, I. Pali-Schöll, H. Mangge, E. Jensen-Jarolim, A. Bernkop-Schnürch, R. Prassl, Thiomer-coated liposomes harbor permeation enhancing and efflux pump inhibitory properties, J. Control. Release 165 (2013) 207–215.

[242] K. Gradauer, J. Barthelmes, C. Vonach, G. Almer, H. Mangge, B. Teubl, E. Roblegg, S. Dünnhaupt, E. Fröhlich, A. Bernkop-Schnürch, R. Prassl, Liposomes coated with thiolated chitosan enhance oral peptide delivery to rats, J. Control. Release 172 (2013) 872–878.

[243] D. Sakloetsakun, S. Dünnhaupt, J. Barthelmes, G. Perera, A. Bernkop-Schnürch, Combining two technologies: multifunctional polymers and self-nanoemulsifying drug delivery system (SNEDDS) for oral insulin administration, Int. J. Biol. Macromol. 61 (2013) 363–372.

[244] M.H. Dufresne, M.A. Gauthier, J.C. Leroux, Thiol-functionalized polymeric micelles: from molecular recognition to improved mucoadhesion, Bioconjug. Chem. 16 (2005) 1027–1033.

[245] M. Ijaz, B. Matuszczak, D. Rahmat, A. Mahmood, S. Bonengel, S. Hussain, C.W. Huck, A. Bernkop-Schnürch, Synthesis and characterization of thiolated β-cyclodextrin as a novel mucoadhesive excipient for intra-oral drug delivery, Carbohydr. Polym. 132 (2015) 187–195.
[246] R. Moll, M. Divo, L. Langbein, The human keratins: biology and pathology, Histochem. Cell Biol. 129 (2008) 705–733.
[247] P.W. Wertz, D.T. Downing, Stratum corneum: biological and biochemical considerations, transdermal drug delivery, 35, 1989 1–17.
[248] A. Vasconcelos, G. Freddi, A. Cavaco-Paulo, Biodegradable materials based on silk fibroin and keratin, Biomacromolecules 9 (2008) 1299–1305.
[249] A. Miranda-Vilela, A. Botelho, L. Muehlmann, An overview of chemical straightening of human hair: technical aspects, potential risks to hair fibre and health and legal issues, Int. J. Cosmet. Sci. 36 (2014) 2–11.
[250] C. Popescu, H. Höcker, Hair—the most sophisticated biological composite material, Chem. Soc. Rev. 36 (2007) 1282–1291.
[251] L.J. Wolfram, D.L. Underwood, The equilibrium between the disulfide linkage in hair keratin and sulfite or mercaptan, Text. Res. J. 36 (1966) 947–953.
[252] C. Valenta, A. Walzer, A.E. Clausen, A. Bernkop-Schnürch, Thiolated polymers: development and evaluation of transdermal delivery systems for progesterone, Pharm. Res. 18 (2001) 211–216.
[253] A. Partenhauser, O. Zupancic, J. Rohrer, S. Bonengel, A. Bernkop-Schnürch, Thiolated silicone oils as adhesive skin protectants for improved barrier function, Int. J. Cosmet. Sci. 38 (2016) 257–265.
[254] F. Laffleur, A. Bernkop-Schnürch, Evaluation of dermal adhesive formulations for topical application, Eur. J. Pharm. Biopharm. 124 (2018) 89–94.
[255] F. Laffleur, Evaluation of chemical modified hydrogel formulation for topical suitability, Int. J. Biol. Macromol. 105 (2017) 1310–1314.
[256] P. Zhang, N. Zhang, Q. Wang, P. Wang, J. Yuan, J. Shen, X. Fan, Disulfide bond reconstruction: a novel approach for grafting of thiolated chitosan onto wool, Carbohydr. Polym. 203 (2019) 369–377.
[257] Y. Zhang, S. Zhou, F. Deng, X. Chen, X. Wang, Y. Wang, H. Zhang, W. Dai, B. He, Q. Zhang, X. Wang, The function and mechanism of preactivated thiomers in triggering epithelial tight junctions opening, Eur. J. Pharm. Biopharm. 133 (2018) 188–199.
[258] F.L. Huang, Y.J. Shiao, S.J. Hou, C.N. Yang, Y.J. Chen, C.H. Lin, F.S. Shie, H.J. Tsay, Cysteine-rich domain of scavenger receptor AI modulates the efficacy of surface targeting and mediates oligomeric Aβ internalization, J. Biomed. Sci. 20 (2013) 54.
[259] A. Sebé-Pedrós, A.J. Roger, F.B. Lang, N. King, I. Ruiz-Trillo, Ancient origin of the integrin-mediated adhesion and signaling machinery, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 10142–10147.
[260] A.E. Clausen, C.E. Kast, A. Bernkop-Schnürch, The role of glutathione in the permeation enhancing effect of thiolated polymers, Pharm. Res. 19 (2002) 602–608.
[261] A.G. Torres, M.J. Gait, Exploiting cell surface thiols to enhance cellular uptake, Trends Biotechnol. 30 (2012) 185–190.
[262] G. Digilio, V. Menchise, E. Gianolio, V. Catanzaro, C. Carrera, R. Napolitano, F. Fedeli, S. Aime, Exofacial protein thiols as a route for the internalization of Gd(III)-based complexes for magnetic resonance imaging cell labeling, J. Med. Chem. 53 (2010) 4877–4890.
[263] S. Aubry, F. Burlina, E. Dupont, D. Delaroche, A. Joliot, S. Lavielle, G. Chassaing, S. Sagan, Cell-surface thiols affect cell entry of disulfide-conjugated peptides, FASEB J. 23 (2009) 2956–2967.
[264] V. Grabovac, F. Laffleur, A. Bernkop-Schnürch, Thiomers: Influence of molecular mass and thiol group content of poly(acrylic acid) on efflux pump inhibition, Int. J. Pharm. 493 (2015) 374–379.
[265] F. Moreno-Merlo, T. Nicklee, D. Hedley, Association between tissue hypoxia and elevated non-protein sulphydryl concentrations in human cervical carcinoma xenografts, Br. J. Cancer 81 (1999) 988–989.
[266] J.J. Glass, Y. Li, R. De Rose, A.P. Johnston, E.I. Czuba, S.Y. Khor, J.F. Quinn, M.R. Whittaker, T.P. Davis, S.J. Kent, Thiol-reactive star polymers display enhanced association with distinct human blood components, ACS Appl. Mater. Interfaces 9 (2017) 12182–12194.
[267] T.N. Gevrek, M. Cosar, D. Aydin, E. Kaga, M. Arslan, R. Sanyal, A. Sanyal, Facile fabrication of a modular "catch and release" hydrogel interface: harnessing thiol-disulfide exchange for reversible protein capture and cell attachment, ACS Appl. Mater. Interfaces 10 (2018) 14399–14409.
[268] A. Alexander Ajazuddin, J. Khan, T.K. Giri, D.K. Tripathi, S. Saraf, Advancement in stimuli triggered *in situ* gelling delivery for local and systemic route, Expert. Opin. Drug Deliv. 9 (2012) 1573–1592.
[269] A.G. Sontyana, A.P. Mathew, K.H. Cho, S. Uthaman, I.K. Park, Biopolymeric *in situ* hydrogels for tissue engineering and bioimaging applications, Tissue Eng. Regen. Med. 15 (2018) 575–590.
[270] W. Zhao, M. Kong, C. Feng, X. Cheng, Y. Liu, X. Chen, Investigation of gelling behavior of thiolated chitosan in alkaline condition and its application in stent coating, Carbohydr. Polym. 136 (2016) 307–315.
[271] J. Chen, F. Ye, Y. Zhou, G. Zhao, Thiolated citrus low-methoxyl pectin: synthesis, characterization and rheological and oxidation-responsive gelling properties, Carbohydr. Polym. 181 (2018) 964–973.
[272] M.D. Hornof, C.E. Kast, A. Bernkop-Schnürch, *In vitro* evaluation of the viscoelastic properties of chitosan-thioglycolic acid conjugates, Eur. J. Pharm. Biopharm. 55 (2003) 185–190.
[273] T. Zarembinski, I. Erickson, N. Doty, in: S. United (Ed.), Thiolated hyaluronan-based hydrogels cross-linked using oxidized glutathione, BIO TIME Inc, 2014.
[274] A.E. Mohrman, M. Farrag, R.K. Grimm, N.D. Leipzig, Evaluation of *in situ* gelling chitosan-PEG copolymer for use in the spinal cord, J. Biomater. Appl. 33 (2018) 435–446.
[275] L. Russo, A. Sgambato, R. Visone, P. Occhetta, M. Moretti, M. Rasponi, F. Nicotra, L. Cipolla, Gelatin hydrogels via thiol-ene chemistry, Monatsh. Chem. Chem. Mon. 147 (2016) 587–592.
[276] Z.C. Wang, X.D. Xu, C.S. Chen, L. Yun, J.C. Song, X.Z. Zhang, R.X. Zhuo, *in situ* formation of thermosensitive PNIPAAm-based hydrogels by Michael-type addition reaction, ACS Appl. Mater. Interfaces 2 (2010) 1009–1018.
[277] R. Censi, P.J. Fieten, P. di Martino, W.E. Hennink, T. Vermonden, *In situ* forming hydrogels by tandem thermal gelling and michael addition reaction between thermosensitive triblock copolymers and thiolated hyaluronan, Macromolecules 43 (2010) 5771–5778.
[278] A. Bernkop-Schnürch, S. Scholler, R.G. Biebel, Development of controlled drug release systems based on thiolated polymers, J. Control. Release 66 (2000) 39–48.
[279] M. Hornof, W. Weyenberg, A. Ludwig, A. Bernkop-Schnürch, Mucoadhesive ocular insert based on thiolated poly(acrylic acid): development and *in vivo* evaluation in humans, J. Control. Release 89 (2003) 419–428.
[280] B. Wirostko, Ocular Composition and Method, US2015157563 (A1) 2015.
[281] M. Moreno, P.Y. Pow, T.S.T. Tabitha, S. Nirmal, A. Larsson, K. Radhakrishnan, J. Nirmal, S.T. Quah, S. Geifman Shochat, R. Agrawal, S. Venkatraman, Modulating release of ranibizumab and aflibercept from thiolated chitosan-based hydrogels for potential treatment of ocular neovascularization, Expert. Opin. Drug Deliv. 14 (2017) 913–925.
[282] A. Anitha, N. Deepa, K.P. Chennazhi, V.K. Lakshmanan, R. Jayakumar, Combinatorial anticancer effects of curcumin and 5-fluorouracil loaded thiolated chitosan nanoparticles towards colon cancer treatment, Biochim. Biophys. Acta 1840 (2014) 2730–2743.
[283] F. Meng, W.E. Hennink, Z. Zhong, Reduction-sensitive polymers and bioconjugates for biomedical applications, Biomaterials 30 (2009) 2180–2198.
[284] Y. Kakizawa, A. Harada, K. Kataoka, Environment-sensitive stabilization of core–shell structured polyion complex micelle by reversible cross-linking of the core through disulfide bond, JACS 121 (1999) 11247–11248.
[285] O. Defang, S. Neha, Z. Hong, C.S. Sean, S.P. Harendra, Reducible disulfide-based non-viral gene delivery systems, Mini-Rev. Med. Chem. 9 (2009) 1242–1250.
[286] Y.S. Lee, S.W. Kim, Bioreducible polymers for therapeutic gene delivery, J. Control. Release 190 (2014) 424–439.
[287] J. Huang, Y. Xue, N. Cai, H. Zhang, K. Wen, X. Luo, S. Long, F. Yu, Efficient reduction and pH co-triggered DOX-loaded magnetic nanogel carrier using disulfide crosslinking, Mater. Sci. Eng. C Mater. Biol. Appl. 46 (2015) 41–51.
[288] H. Han, S. Son, S. Son, N. Kim, J.Y. Yhee, J.H. Lee, J.S. Choi, C.K. Joo, H. Lee, D. Lee, W.J. Kim, S.H. Kim, I.C. Kwon, H. Kim, K. Kim, Reducible polyethylenimine nanoparticles for efficient sirna delivery in corneal neovascularization therapy, Macromol. Biosci. 16 (2016) 1583–1597.
[289] M. Muthiah, H.L. Che, S. Kalash, J. Jo, S.Y. Choi, W.J. Kim, C.S. Cho, J.Y. Lee, I.K. Park, Formulation of glutathione responsive anti-proliferative nanoparticles from thiolated Akt1 siRNA and disulfide-crosslinked PEI for efficient anti-cancer gene therapy, Colloids Surf. B: Biointerfaces 126 (2015) 322–327.
[290] L.V. Christensen, C.W. Chang, W.J. Kim, S.W. Kim, Z. Zhong, C. Lin, J.F. Engbersen, J. Feijen, Reducible poly(amido ethylenimine)s designed for triggered intracellular gene delivery, Bioconjug. Chem. 17 (2006) 1233–1240.
[291] L.V. Christensen, C.W. Chang, J.W. Yockman, R. Conners, H. Jackson, Z. Zhong, J. Feijen, D.A. Bull, S.W. Kim, Reducible poly(amido ethylenediamine) for hypoxia-inducible VEGF delivery, J. Control. Release 118 (2007) 254–261.
[292] A.N. McGinn, H.Y. Nam, M. Ou, C.M. Straub, N. Hu, J.W. Yockman, D.A. Bull, S.W. Kim, Bioreducible polymer-transfected skeletal myoblasts for vegf delivery to acutely ischemic myocardium, Biomaterials 32 (2011) 942–949.
[293] J. Beloor, C.S. Choi, H.Y. Nam, M. Park, S.H. Kim, A. Jackson, K.Y. Lee, S.W. Kim, P. Kumar, S.K. Lee, Arginine-engrafted biodegradable polymer for the systemic delivery of therapeutic siRNA, Biomaterials 33 (2012) 1640–1650.
[294] W. Wang, H. Sun, F. Meng, S. Ma, H. Liu, Z. Zhong, Precise control of intracellular drug release and anti-tumor activity of biodegradable micellar drugs via reduction-sensitive shell-shedding, Soft Matter 8 (2012) 3949–3956.
[295] H. Sun, B. Guo, R. Cheng, F. Meng, H. Liu, Z. Zhong, Biodegradable micelles with sheddable poly(ethylene glycol) shells for triggered intracellular release of doxorubicin, Biomaterials 30 (2009) 6358–6366.
[296] J. Liu, Y. Pang, W. Huang, X. Huang, L. Meng, X. Zhu, Y. Zhou, D. Yan, Bioreducible micelles self-assembled from amphiphilic hyperbranched multiarm copolymer for glutathione-mediated intracellular drug delivery, Biomacromolecules 12 (2011) 1567–1577.
[297] E. Verheyen, S. van der Wal, H. Deschout, K. Braeckmans, S. de Smedt, A. Barendregt, W.E. Hennink, C.F. van Nostrum, Protein macromonomers containing reduction-sensitive linkers for covalent immobilization and glutathione triggered release from dextran hydrogels, J. Control. Release 156 (2011) 329–336.
[298] L. Brülisauer, M.A. Gauthier, J.C. Leroux, Disulfide-containing parenteral delivery systems and their redox-biological fate, J. Control. Release 195 (2014) 147–154.
[299] C. Müller, B.N. Ma, R. Gust, A. Bernkop-Schnürch, Thiopyrazole preactivated chitosan: combining mucoadhesion and drug delivery, Acta Biomater. 9 (2013) 6585–6593.
[300] K. Albrecht-Schgoer, J. Barthelmes, W. Schgoer, M. Theurl, I. Nardin, D. Lener, C. Gutmann, S. Dünnhaupt, A. Bernkop-Schnürch, R. Kirchmair, Nanoparticular delivery system for a secretoneurin derivative induces angiogenesis in a hind limb ischemia model, J. Control. Release 250 (2017) 1–8.
[301] Y. Chen, X. Liu, R. Liu, Y. Gong, M. Wang, Q. Huang, Q. Feng, B. Yu, Zero-order controlled release of BMP2-derived peptide P24 from the chitosan scaffold by chemical grafting modification technique for promotion of osteogenesis, Theranostics 7 (2017) 1072–1087.

[302] M.D. Roth, A. Harui, Modified hyaluronic acid hydrogels and proteins for the time-controlled release of biologic agents, O. World Intellectual Property (Ed.), The Regents Of The University Of California, 2017.

[303] Y. Lu, A. Shah, R.A. Hunter, R.J. Soto, M.H. Schoenfisch, S-Nitrosothiol-modified nitric oxide-releasing chitosan oligosaccharides as antibacterial agents, Acta Biomater. 12 (2015) 62–69.

[304] A. Pegalajar-Jurado, K.A. Wold, J.M. Joslin, B.H. Neufeld, K.A. Arabea, L.A. Suazo, S.L. McDaniel, R.A. Bowen, M.M. Reynolds, Reprint of: Nitric oxide-releasing polysaccharide derivative exhibits 8-log reduction against Escherichia coli, Acinetobacter baumannii and Staphylococcus aureus, J. Control. Release 220 (2015) 617–623.

[305] M.C. Frost, M.E. Meyerhoff, Synthesis, characterization, and controlled nitric oxide release from S-nitrosothiol-derivatized fumed silica polymer filler particles, J. Biomed. Mater. Res. Part A 72 (2005) 409–419.

[306] H. Katsumi, M. Nishikawa, F. Yamashita, M. Hashida, Development of polyethylene glycol-conjugated poly-S-nitrosated serum albumin, a novel S-Nitrosothiol for prolonged delivery of nitric oxide in the blood circulation *in vivo*, J. Pharmacol. Exp. Ther. 314 (2005) 1117–1124.

[307] N.A. Stasko, T.H. Fischer, M.H. Schoenfisch, S-nitrosothiol-modified dendrimers as nitric oxide delivery vehicles, Biomacromolecules 9 (2008) 834–841.

[308] V.B. Damodaran, L.W. Place, M.J. Kipper, M.M. Reynolds, Enzymatically degradable nitric oxide releasing S-nitrosated dextran thiomers for biomedical applications, J. Mater. Chem. 22 (2012) 23038–23048.

[309] N. Langoth, A. Bernkop-Schnürch, P. Kurka, *In vitro* evaluation of various buccal permeation enhancing systems for PACAP (pituitary adenylate cyclase-activating polypeptide), Pharm. Res. 22 (2005) 2045–2050.

[310] A. Bernkop-Schnürch, C.E. Kast, D. Guggi, Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: thiomer/GSH systems, J. Control. Release 93 (2003) 95–103.

[311] X. Wang, J. Iqbal, D. Rahmat, A. Bernkop-Schnürch, Preactivated thiomers: permeation enhancing properties, Int. J. Pharm. 438 (2012) 217–224.

[312] G. Di Colo, Y. Zambito, C. Zaino, Polymeric enhancers of mucosal epithelia permeability: synthesis, transepithelial penetration-enhancing properties, mechanism of action, safety issues, J. Pharm. Sci. 97 (2008) 1652–1680.

[313] X. Wang, C. Zheng, Z. Wu, D. Teng, X. Zhang, Z. Wang, C. Li, Chitosan-NAC nanoparticles as a vehicle for nasal absorption enhancement of insulin, J Biomed Mater Res B Appl Biomater 88 (2009) 150–161.

[314] D. Singh, M. Rashid, S.S. Hallan, N.K. Mehra, A. Prakash, N. Mishra, Pharmacological evaluation of nasal delivery of selegiline hydrochloride-loaded thiolated chitosan nanoparticles for the treatment of depression, artificial cells, Nanomedicine Biotechnol. 44 (2016) 865–877.

[315] N. Langoth, H. Kahlbacher, G. Schöffmann, I. Schmerold, M. Schuh, S. Franz, P. Kurka, A. Bernkop-Schnürch, Thiolated chitosans: design and *in vivo* evaluation of a mucoadhesive buccal peptide drug delivery system, Pharm. Res. 23 (2006) 573–579.

[316] M.M. Gottesman, I. Pastan, The multidrug transporter, a double-edged sword, J. Biol. Chem. 263 (1988) 12163–12166.

[317] F.J. Sharom, ABC multidrug transporters: structure, function and role in chemoresistance, Pharmacogenomics 9 (2008) 105–127.

[318] A. Trapani, C. Palazzo, M. Contino, M.G. Perrone, N. Cioffi, N. Ditaranto, N.A. Colabufo, M. Conese, G. Trapani, G. Puglisi, Mucoadhesive properties and interaction with P-glycoprotein (P-gp) of thiolated-chitosans and -glycol chitosans and corresponding parent polymers: a comparative study, Biomacromolecules 15 (2014) 882–893.

[319] F. Föger, H. Hoyer, K. Kafedjiiski, M. Thaurer, A. Bernkop-Schnürch, *In vivo* comparison of various polymeric and low molecular mass inhibitors of intestinal P-glycoprotein, Biomaterials 27 (2006) 5855–5860.

[320] V. Grabovac, F. Laffleur, A. Bernkop-Schnürch, Thiomers: influence of molecular mass and thiol group content of poly(acrylic acid) on efflux pump inhibition, Int. J. Pharm. 493 (2015) 374–379.

[321] F. Föger, H. Hoyer, K. Kafedjiiski, M. Thaurer, A. Bernkop-Schnürch, *In vivo* comparison of various polymeric and low molecular mass inhibitors of intestinal P-glycoprotein, Biomaterials 27 (2006) 5855–5860.

[322] F. Föger, T. Schmitz, A. Bernkop-Schnürch, *In vivo* evaluation of an oral delivery system for P-gp substrates based on thiolated chitosan, Biomaterials 27 (2006) 4250–4255.

[323] F. Föger, S. Malaivijitnond, T. Wannaprasert, C. Huck, A. Bernkop-Schnürch, M. Werle, Effect of a thiolated polymer on oral paclitaxel absorption and tumor growth in rats, J. Drug Target. 16 (2008) 149–155.

[324] K. Netsomboon, F. Laffleur, W. Suchaoin, A. Bernkop-Schnürch, Novel *in vitro* transport method for screening the reversibility of P-glycoprotein inhibitors, Eur. J. Pharm. Biopharm. 100 (2016) 9–14.

[325] J. Smirnova, E. Kabin, I. Järving, O. Bragina, V. Tõugu, T. Plitz, P. Palumaa, Copper(I)-binding properties of de-coppering drugs for the treatment of Wilson disease. α-Lipoic acid as a potential anti-copper agent, Sci. Rep. 8 (2018) 1463.

[326] A. Bernkop-Schnürch, S.C. Thaler, Polycarbophil–cysteine conjugates as platforms for oral polypeptide delivery systems, J. Pharm. Sci. 89 (2000) 901–909.

[327] F. Madsen, N.A. Peppas, Complexation graft copolymer networks: swelling properties, calcium binding and proteolytic enzyme inhibition, Biomaterials 20 (1999) 1701–1708.

[328] D. Ameye, J. Voorspoels, P. Foreman, J. Tsai, P. Richardson, S. Geresh, J.P. Remon, Trypsin inhibition, calcium and zinc ion binding of starch–g–poly(acrylic acid) copolymers and starch/poly(acrylic acid) mixtures for peroral peptide drug delivery, J. Control. Release 75 (2001) 357–364.

[329] C. Valenta, M. Marschütz, C. Egyed, A. Bernkop-Schnürch, Evaluation of the inhibition effect of thiolated poly(acrylates) on vaginal membrane bound aminopeptidase N and release of the model drug LH-RH, J. Pharm. Pharmacol. 54 (2002) 603–610.

[330] A. Bernkop-Schnürch, H. Zarti, G.F. Walker, Thiolation of polycarbophil enhances its inhibition of intestinal brush border membrane bound aminopeptidase N, J. Pharm. Sci. 90 (2001) 1907–1914.

[331] I. Bravo-Osuna, G. Millotti, C. Vauthier, G. Ponchel, *In vitro* evaluation of calcium binding capacity of chitosan and thiolated chitosan poly(isobutyl cyanoacrylate) core–shell nanoparticles, Int. J. Pharm. 338 (2007) 284–290.

[332] P. Singh, K. Chauhan, V. Priyaa, S. R.K., A greener approach for impressive removal of As(III)/As(V) from an ultra-low concentration using a highly efficient chitosan thiomer as a new adsorbent, RSC Adv. 6 (2016) 64946–64961.

[333] M.H. Stewart, K. Susumu, B.C. Mei, I.L. Medintz, J.B. Delehanty, J.B. Blanco-Canosa, P.E. Dawson, H. Mattoussi, Multidentate Poly(ethylene glycol) Ligands Provide Colloidal Stability to Semiconductor and Metallic Nanocrystals in Extreme Conditions, J. Am. Chem. Soc. 132 (2010) 9804–9813.

[334] B.C. Mei, K. Susumu, I.L. Medintz, J.B. Delehanty, T.J. Mountziaris, H. Mattoussi, Modular poly(ethylene glycol) ligands for biocompatible semiconductor and gold nanocrystals with extended pH and ionic stability, J. Mater. Chem. 18 (2008) 4949–4958.

[335] D.-H. Tsai, F.W. DelRio, R.I. MacCuspie, T.J. Cho, M.R. Zachariah, V.A. Hackley, Competitive adsorption of thiolated polyethylene glycol and mercaptopropionic acid on gold nanoparticles measured by physical characterization methods, Langmuir 26 (2010) 10325–10333.

[336] S. Roux, B. Garcia, J.-L. Bridot, M. Salomé, C. Marquette, L. Lemelle, P. Gillet, L. Blum, P. Perriat, O. Tillement, Synthesis, characterization of dihydrolipoic acid capped gold nanoparticles, and functionalization by the electroluminescent luminol, Langmuir 21 (2005) 2526–2536.

[337] H. Lee, S.H. Choi, T.G. Park, Direct visualization of hyaluronic acid polymer chain by self-assembled one-dimensional array of gold nanoparticles, Macromolecules 39 (2006) 23–25.

[338] A. Partenhauser, A. Bernkop-Schnürch, Mucoadhesive polymers in the treatment of dry X syndrome, Drug Discov. Today 21 (2016) 1051–1062.

[339] M. Prinz, Aqueous ophthalmic solution and method for treating dry eye syndrome, in: S. United (Ed.), Croma-Pharma GmbH, 2017.

[340] M. Prinz, S. Holler, Therapeutic use of a sterile aqueous ophthalmic solution, in: U. European (Ed.), Croma-Pharma GmbH, Croma Pharma GmbH, 2018.

[341] D. Schmidl, R. Werkmeister, S. Kaya, A. Unterhuber, K.J. Witkowska, R. Baumgartner, S. Holler, M. O'Rourke, W. Peterson, A. Wolter, M. Prinz, L. Schmetterer, G. Garhofer, A Controlled, randomized double-blind study to evaluate the safety and efficacy of chitosan-N-acetylcysteine for the treatment of dry eye syndrome, J. Ocul. Pharmacol. Ther. 33 (2017) 375–382.

[342] M. Messina, H.S. Dua, Early results on the use of chitosan-N-acetylcysteine (Lacrimera®) in the management of dry eye disease of varied etiology, Int. Ophthalmol. 39 (2019) 693–696.

[343] S. Hoeller, M. Hornof, L. Schmetterer, G. Garhoefer, Safety and tolerability of chitosan-n-acetylcysteine eye drops in healthy young volunteers, Investig. Ophthalmol. Vis. Sci. 52 (2011) 3841.

[344] M. Messina, H.S. Dua, Early results on the use of chitosan-N-acetylcysteine (Lacrimera®) in the management of dry eye disease of varied etiology, Int. Ophthalmol. 39 (2019) 693–696.

[345] K. Lorenz, G. Garhofer, S. Hoeller, W. Peterson, R.M. Vielnascher, Z.I. Schoenfeld, M. Prinz, Long-term management of dry eye by once-daily use of Chitosan-N-Acetylcysteine (Lacrimera®) eye drops, J. Clin. Ophthalmol. 2 (2018) 47–54.

[346] Safety and Tolerability of Chitosan-N-acetylcysteine Eye Drops in Healthy Young Volunteers, in https://clinicaltrials.gov/ct2/show/NCT01015209?term=chitosan&draw=3&rank=17.

[347] Local Tolerability of Chitosan-N-acetylcysteine, in https://clinicaltrials.gov/ct2/show/NCT01278784?term=chitosan&rank=4.

[348] German Innovation Award 2018, , in https://www.german-innovation-award.de/en/winners/preis/gewinner/lacrimerar/.

[349] D.L. Williams, B.K. Mann, Efficacy of a crosslinked hyaluronic acid-based hydrogel as a tear film supplement: a masked controlled study, PLoS ONE 9 (2014), e99766.

[350] C. Cagini, G. Torroni, T. Fiore, A. Cerquaglia, M. Lupidi, P. Aragona, B. Iaccheri, Tear film stability in sjögren syndrome patients treated with hyaluronic acid versus crosslinked hyaluronic acid-based eye drops, J. Ocul. Pharmacol. Ther. 33 (2017) 539–542.

[351] A. Partenhauser, K. Netsomboon, I. Pereira de Sousa, F. Laffleur, A. Bernkop-Schnürch, Preactivated silicone oil as potential long-term vitreous replacement with nonemulsifying properties, J Biomed Mater Res B Appl Biomater 105 (2017) 551–559.

[352] Safety of Hyaluronan Thiomer i.o, Implant During Combined Phacoemulsification - Non Penetrating Deep Sclerectomy, https://clinicaltrials.gov/ct2/show/study/NCT01887873

[353] D.S. Durrie, D. Wolsey, V. Thompson, C. Assang, B. Mann, B. Wirostko, Ability of a new crosslinked polymer ocular bandage gel to accelerate reepithelialization after photorefractive keratectomy, J Cataract Refract Surg 44 (2018) 369–375.

[354] Y. Liu, S. Ahmad, X.Z. Shu, R.K. Sanders, S.A. Kopesec, G.D. Prestwich, Accelerated repair of cortical bone defects using a synthetic extracellular matrix to deliver human demineralized bone matrix, J. Orthop. Res. 24 (2006) 1454–1462.

[355] I.H. Bae, B.C. Jeong, M.S. Kook, S.H. Kim, J.T. Koh, Evaluation of a thiolated chitosan scaffold for local delivery of BMP-2 for osteogenic differentiation and ectopic bone formation, Biomed. Res. Int. 2013 (2013) 878930.

[356] K. Chauhan, R. Sharma, R. Dharela, G.S. Chauhan, R.K. Singhal, Chitosan-thiomer stabilized silver nano-composites for antimicrobial and antioxidant applications, RSC Adv. 6 (2016) 75453–75464.

[357] L. Coppo, P. Ghezzi, Thiol regulation of pro-inflammatory cytokines and innate immunity: protein S-thiolation as a novel molecular mechanism, Biochem. Soc. Trans. 39 (2011) 1268–1272.

[358] F. Zahir-Jouzdani, M. Mahbod, M. Soleimani, F. Vakhshiteh, E. Arefian, S. Shahosseini, R. Dinarvand, F. Atyabi, Chitosan and thiolated chitosan: novel therapeutic approach for preventing corneal haze after chemical injuries, Carbohydr. Polym. 179 (2018) 42–49.

[359] D.-W. Lee, S.A. Shirley, R.F. Lockey, S.S. Mohapatra, Thiolated chitosan nanoparticles enhance anti-inflammatory effects of intranasally delivered theophylline, Respir. Res. 7 (2006) 112.

[360] Y. Zhao, T.D. Biggs, M. Xian, Hydrogen sulfide (H2S) releasing agents: chemistry and biological applications, Chem. Commun. (Camb.) 50 (2014) 11788–11805.

[361] M. Prinz, M. Hoffer, Hydrogen Sulfide Releasing Polymer Compounds, WO2018083326 (A1) 2018.

[362] S.H. Yu, F. Ercole, N.A. Veldhuis, M.R. Whittaker, D.T.P.J.F. Quinn, Polymers with acyl-protected perthiol chain termini as convenient building blocks for doubly responsive H2S-donating nanoparticles, Polym. Chem. (2017) 41.

[363] Y. Zhao, S. Bhushan, C. Yang, H. Otsuka, J.D. Stein, A. Pacheco, B. Peng, N.O. Devarie-Baez, H.C. Aguilar, D.J. Lefer, M. Xian, Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury, ACS Chem. Biol. 8 (2013) 1283–1290.

[364] A. Jain, P. Hurkat, A. Jain, A. Jain, A. Jain, S.K. Jain, Thiolated polymers: pharmaceutical tool in nasal drug delivery of proteins and peptides, Int. J. Pept. Res. Therap. 25 (2019) 15–26.

[365] M. Hanif, M. Zaman, S. Qureshi, Thiomers: a blessing to evaluating era of pharmaceuticals, Int. J. Polym. Sci. (2015) 1–9.

[366] D. Jere, R. Arote, H.L. Jiang, Y.K. Kim, M.H. Cho, C.S. Cho, Bioreducible polymers for efficient gene and siRNA delivery, Biomed. Mater. 4 (2009), 025020.

[367] W. Samprasit, R. Kaomongkolgit, M. Sukma, T. Rojanarata, T. Ngawhirunpat, P. Opanasopit, Mucoadhesive electrospun chitosan-based nanofibre mats for dental caries prevention, Carbohydr. Polym. 117 (2015) 933–940.

[368] A.A. Kassem, D.A. Issa, G.S. Kotry, R.M. Farid, Thiolated alginate-based multiple layer mucoadhesive films of metformin forintra-pocket local delivery: *in vitro* characterization and clinical assessment, Drug Dev. Ind. Pharm. 43 (2017) 120–131.

[369] G.S. Kotry, R.M. Farid, A. Kassem, D.A.E. Issa, Clinical and Radiographic assessment of the adjunctive intra-pocket application of triple-layer mucoadhesive metformin film in non-surgical management of chronic periodontitis, in, 2016.

[370] J. Gao, X. Huang, H. Liu, F. Zan, J. Ren, Colloidal stability of gold nanoparticles modified with thiol compounds: bioconjugation and application in cancer cell imaging, Langmuir 28 (2012) 4464–4471.

[371] E. Hutter, D. Maysinger, Gold nanoparticles and quantum dots for bioimaging, Microsc. Res. Tech. 74 (2011) 592–604.

[372] P. F. Jiao, H. Y. Zhou, L. X. Chen, B. Yan, Cancer-targeting multifunctionalized gold nanoparticles in imaging and therapy, Curr. Med. Chem. 18 (2011) 2086–2102.

[373] X. Huang, M.A. El-Sayed, Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy, J. Adv. Res. 1 (2010) 13–28.

[374] W. Cai, T. Gao, H. Hong, J. Sun, Applications of gold nanoparticles in cancer nanotechnology, Nanotechnol. Sci. Appl. 1 (2008) 17.

[375] X. Li, L. Xing, K. Zheng, P. Wei, L. Du, M. Shen, X. Shi, Formation of gold nanostar-coated hollow mesoporous silica for tumor multimodality imaging and photothermal therapy, ACS Appl. Mater. Interfaces 9 (2017) 5817–5827.

[376] J.M. Bergen, H.A. von Recum, T.T. Goodman, A.P. Massey, S.H. Pun, Gold nanoparticles as a versatile platform for optimizing physicochemical parameters for targeted drug delivery, Macromol. Biosci. 6 (2006) 506–516.

[377] C. Chattaway, S. Belbekhouche, F.E. Du Prez, K. Glinel, S. Demoustier-Champagne, Bifunctionalized redox-responsive layers prepared from a thiolactone copolymer, Langmuir 34 (2018) 5234–5244.

[378] W. Eck, G. Craig, A. Sigdel, G. Ritter, L.J. Old, L. Tang, M.F. Brennan, P.J. Allen, M.D. Mason, PEGylated gold nanoparticles conjugated to monoclonal F19 antibodies as targeted labeling agents for human pancreatic carcinoma tissue, ACS Nano 2 (2008) 2263–2272.

[379] G.F. Paciotti, L. Myer, D. Weinreich, D. Goia, N. Pavel, R.E. McLaughlin, L. Tamarkin, Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery, Drug deliv. 11 (2004) 169–183.

[380] A. Sood, V. Arora, J. Shah, R.K. Kotnala, T.K. Jain, Multifunctional gold coated iron oxide core-shell nanoparticles stabilized using thiolated sodium alginate for biomedical applications, Mater. Sci. Eng. 80 (2017) 274–281.

[381] V. Arora, A. Sood, J. Shah, R.K. Kotnala, T.K. Jain, Synthesis and characterization of thiolated pectin stabilized gold coated magnetic nanoparticles, Mater. Chem. Phys. 173 (2016) 161–167.

[382] A. Vetter, A. Reinisch, D. Strunk, C. Kremser, H.W. Hahn, C.W. Huck, T. Ostermann, K. Leithner, A. Bernkop-Schnürch, Thiolated polyacrylic acid-modified iron oxide nanoparticles for *in vitro* labeling and MRI of stem cells, J. Drug Target. 19 (2011) 562–572.

[383] D. Li, X. Tang, B. Pulli, C. Lin, P. Zhao, J. Cheng, Z. Lv, X. Yuan, Q. Luo, H. Cai, M. Ye, Theranostic nanoparticles based on bioreducible polyethylenimine-coated iron oxide for reduction-responsive gene delivery and magnetic resonance imaging, Int. J. Nanomedicine 9 (2014) 3347–3361.

[384] G. Shahnaz, C. Kremser, A. Reinisch, A. Vetter, F. Laffleur, D. Rahmat, J. Iqbal, S. Dünnhaupt, W. Salvenmoser, R. Tessadri, U. Griesser, A. Bernkop-Schnürch, Efficient MRI labeling of endothelial progenitor cells: design of thiolated surface stabilized superparamagnetic iron oxide nanoparticles, Eur. J. Pharm. Biopharm. 85 (2013) 346–355.

[385] R.J. Soto, B.J. Privett, M.H. Schoenfisch, *In vivo* analytical performance of nitric oxide-releasing glucose biosensors, Anal. Chem. 86 (2014) 7141–7149.

[386] R.J. Soto, J.B. Schofield, S.E. Walter, M.J. Malone-Povolny, M.H. Schoenfisch, Design considerations for Silica-Particle-Doped Nitric-Oxide-Releasing polyurethane glucose biosensor membranes, ACS Sensors 2 (2017) 140–150.

[387] C. Fischak, R. Klaus, R.M. Werkmeister, C. Hohenadl, M. Prinz, L. Schmetterer, G. Garhöfer, Effect of topically administered chitosan, J. Ophthalmol. (2017) (2017), 5192924.

[388] B. Wirostko, B.K. Mann, D.L. Williams, G.D. Prestwich, Ophthalmic uses of a thiol-modified hyaluronan-based hydrogel, Adv. Wound Care (New Rochelle) 3 (2014) 708–716.

[389] L. Espandar, B. Bunnell, G. Wang, P. Gregory, C. McBride, M. Moshirfar, Adipose-derived stem cells on hyaluronic acid–derived scaffold: a new horizon in bioengineered cornea, Arch. Ophthalmol. 130 (2012) 202–208.

[390] S. Schnichels, N. Schneider, C. Hohenadl, J. Hurst, A. Schatz, K. Januschowski, M.S. Spitzer, Efficacy of two different thiol-modified crosslinked hyaluronate formulations as vitreous replacement compared to silicone oil in a model of retinal detachment, PLoS ONE 12 (2017), e0172895.

[391] C. Bauer, V. Jeyakumar, E. Niculescu-Morzsa, D. Kern, S. Nehrer, Hyaluronan thiomer gel/matrix mediated healing of articular cartilage defects in New Zealand white rabbits-a pilot study, J. Exp. Orthop. 4 (2017) 14.

[392] D.L. Williams, B.M. Wirostko, G. Gum, B.K. Mann, Topical cross-linked ha-based hydrogel accelerates closure of corneal epithelial defects and repair of stromal ulceration in Companion Animals, Invest. Ophthalmol. Vis. Sci. 58 (2017) 4616–4622.

[393] G. Yang, G.D. Prestwich, B.K. Mann, Thiolated carboxymethyl-hyaluronic-Acid-based biomaterials enhance wound healing in rats, dogs, and horses, ISRN Vet. Sci. 2011 (2011), 851593.

[394] J.M. Cohen, L.L. Southwood, J. Engiles, M. Leitch, D.M. Nunamaker, Effects of a novel hydrogel on equine bone healing: A pilot study, Vet. Comp. Orthop. Traumatol. 25 (2012) 184–191.

[395] Assessment of Safety and Tolerability of Chitosan-N-acetylcysteine Eye Drops in Subjects While Wearing Contact Lenses and Before Insertion of Contact Lenses, in https://clinicaltrials.gov/ct2/show/NCT01747616?term=chitosan&draw=2&rank=15.

[396] S. Hoeller, M. Hornof, L. Schmetterer, G. Garhoefer, Safety and tolerability of chitosan-N-acetylcysteine eye drops in healthy young volunteers, Investig. Ophtalmol. Visual Sci. 52 (2011) 3841.

[397] Safety of Hyaluronan Thiomer i.o. Implant During Combined Phacoemulsification - Non Penetrating Deep Sclerectomy, in https://clinicaltrials.gov/ct2/show/NCT01887873?term=hyaluronan&rank=12 2013.

[398] C.E. Kast, W. Frick, B. Kapeller, H. Eberl, E. Falkner, K. Macfelda, A. Bernkop-Schnürch, Chitosan-thioglycolic acid conjugate as scaffold in tissue engineering, 4th Central European Symposium on Pharmaceutical Technology, University of Vienna, 2001.

[399] C.E. Kast, W. Frick, U. Losert, A. Bernkop-Schnürch, Chitosan-thioglycolic acid conjugate: a new scaffold material for tissue engineering? Int. Aust. J. Pharm. 256 (2003) 183–189 Proceedings Central European Symposium on Pharmaceutical Technology, Vienna, 2001.

[400] M. Gajendiran, J.S. Rhee, K. Kim, Recent developments in thiolated polymeric hydrogels for tissue engineering applications, Tissue Eng. Part B Rev. 24 (2018) 66–74.

[401] J.L. Vanderhooft, M. Alcoutlabi, J.J. Magda, G.D. Prestwich, Rheological properties of cross-linked hyaluronan-gelatin hydrogels for tissue engineering, Macromol. Biosci. 9 (2009) 20–28.

[402] M.N. Gonzalez, E.J. Tijsma, N. Schaffhausen, Thiolated chitosan gel, in: O. World Intellectual Property (Ed.), Medtronic, Inc, 2009.

[403] F.G.L. Medeiros Borsagli, I.C. Carvalho, H.S. Mansur, Amino acid-grafted and N-acylated chitosan thiomers: Construction of 3D bio-scaffolds for potential cartilage repair applications, Int. J. Biol. Macromol. 114 (2018) 270–282.

[404] Y. Cao, B.H. Lee, H.B. Peled, S.S. Venkatraman, Synthesis of stiffness-tunable and cell-responsive Gelatin-poly(ethylene glycol) hydrogel for three-dimensional cell encapsulation, J. Biomed. Mater. Res.Part A 104 (2016) 2401–2411.

[405] S.S. Shah, M. Kim, K. Cahill-Thompson, G. Tae, A. Revzin, Micropatterning of bioactive heparin-based hydrogels, Soft Matter 7 (2011) 3133–3140.

[406] B.V. Sridhar, E.A. Dailing, J.L. Brock, J.W. Stansbury, M.A. Randolph, K.S. Anseth, A biosynthetic scaffold that facilitates chondrocyte-mediated degradation and promotes articular cartilage extracellular matrix deposition, Regen. Eng. Transl. Med. 1 (2015) 11–21.

[407] A.J. Neumann, T. Quinn, S.J. Bryant, Nondestructive evaluation of a new hydrolytically degradable and photo-clickable PEG hydrogel for cartilage tissue engineering, Acta Biomater. 39 (2016) 1–11.

[408] C. Vogt, Q. Xing, W. He, B. Li, M.C. Frost, F. Zhao, Fabrication and characterization of a nitric oxide-releasing nanofibrous gelatin matrix, Biomacromolecules 14 (2013) 2521–2530.

[409] J.C. Grim, T.E. Brown, B.A. Aguado, D.A. Chapnick, A.L. Viert, X. Liu, K.S. Anseth, A reversible and repeatable thiol-ene bioconjugation for dynamic patterning of signaling proteins in hydrogels, ACS Cent. Sci. 4 (2018) 909–916.

[410] A. Skardal, S.F. Sarker, A. Crabbe, C.A. Nickerson, G.D. Prestwich, The generation of 3-D tissue models based on hyaluronan hydrogel-coated microcarriers within a rotating wall vessel bioreactor, Biomaterials 31 (2010) 8426–8435.

[411] G.D. Prestwich, A. Skardal, J. Zhang, Hydrogels crosslinked with gold nanoparticles and methods of making and using thereof, O. World Intellectual Property (Ed.), University Of Utah Research Foundation, 2010.

[412] A. Skardal, J. Zhang, L. McCoard, S. Oottamasathien, G.D. Prestwich, Dynamically crosslinked gold nanoparticle - hyaluronan hydrogels, Adv. Mater. (Deerfield Beach, Fla.) 22 (2010) 4736–4740.

[413] S. Stichler, T. Jungst, M. Schamel, I. Zilkowski, M. Kuhlmann, T. Bock, T. Blunk, J. Tessmar, J. Groll, Thiol-ene clickable poly(glycidol) hydrogels for biofabrication, Ann. Biomed. Eng. 45 (2017) 273–285.

[414] A. Hoffmann, H. Leonards, N. Tobies, L. Pongratz, K. Kreuels, F. Kreimendahl, C. Apel, M. Wehner, N. Nottrodt, New stereolithographic resin providing functional surfaces for biocompatible three-dimensional printing, J. Tissue Eng. 8 (2017), 2041731417744485.

[415] Y. Liu, X.Z. Shu, G.D. Prestwich, Osteochondral defect repair with autologous bone marrow-derived mesenchymal stem cells in an injectable, *in situ*, cross-linked synthetic extracellular matrix, Tissue Eng. 12 (2006) 3405–3416.

[416] M.K. Nguyen, O. Jeon, M.D. Krebs, D. Schapira, E. Alsberg, Sustained localized presentation of RNA interfering molecules from *in situ* forming hydrogels to guide stem cell osteogenic differentiation, Biomaterials 35 (2014) 6278–6286.

[417] S. Duflo, S.L. Thibeault, W. Li, X.Z. Shu, G.D. Prestwich, Vocal fold tissue repair *in vivo* using a synthetic extracellular matrix, Tissue Eng. 12 (2006) 2171–2180.

[418] C. Bauer, M. Berger, R.R. Baumgartner, S. Holler, H. Zwickl, E. Niculescu-Morzsa, F. Halbwirth, S. Nehrer, A novel cross-linked hyaluronic acid porous scaffold for cartilage repair: an *in vitro* study with osteoarthritic chondrocytes, Cartilage 7 (2016) 265–273.

[419] X. Zheng Shu, Y. Liu, F.S. Palumbo, Y. Luo, G.D. Prestwich, *In situ* crosslinkable hyaluronan hydrogels for tissue engineering, Biomaterials 25 (2004) 1339–1348.

[420] E.M. Horn, M. Beaumont, X.Z. Shu, A. Harvey, G.D. Prestwich, K.M. Horn, A.R. Gibson, M.C. Preul, A. Panitch, Influence of cross-linked hyaluronic acid hydrogels on neurite outgrowth and recovery from spinal cord injury, J. Neurosurg. Spine 6 (2007) 133–140.

[421] P. Moshayedi, L.R. Nih, I.L. Llorente, A.R. Berg, J. Cinkornpumin, W.E. Lowry, T. Segura, S.T. Carmichael, Systematic optimization of an engineered hydrogel allows for selective control of human neural stem cell survival and differentiation after transplantation in the stroke brain, Biomaterials 105 (2016) 145–155.

[422] L.R. Nih, P. Moshayedi, I.L. Llorente, A.R. Berg, J. Cinkornpumin, W.E. Lowry, T. Segura, S.T. Carmichael, Engineered HA hydrogel for stem cell transplantation in the brain: Biocompatibility data using a design of experiment approach, Data Brief. 10 (2017) 202–209.

[423] K. Cheng, A. Blusztajn, D. Shen, T.-S. Li, B. Sun, G. Galang, T.I. Zarembinski, G.D. Prestwich, E. Marbán, R.R. Smith, L. Marbán, Functional performance of human cardiosphere-derived cells delivered in an *in situ* polymerizable hyaluronan-gelatin hydrogel, Biomaterials 33 (2012) 5317–5324.

[424] K. Gwon, E. Kim, G. Tae, Heparin-hyaluronic acid hydrogel in support of cellular activities of 3D encapsulated adipose derived stem cells, Acta Biomater. 49 (2017) 284–295.

[425] Y. Dong, S. A, M. Rodrigues, X. Li, S.H. Kwon, N. Kosaric, S. Khong, Y. Gao, W. Wang, G.C. Gurtner, Injectable and tunable gelatin hydrogels enhance stem cell retention and improve cutaneous wound healing, Adv. Funct. Mater. 27 (2017), 1606619.

[426] S.-W. Wu, X. Liu, A.L. Miller, Y.-S. Cheng, M.-L. Yeh, L. Lu, Strengthening injectable thermo-sensitive NIPAAm-g-chitosan hydrogels using chemical cross-linking of disulfide bonds as scaffolds for tissue engineering, Carbohydr. Polym. 192 (2018) 308–316.

[427] M. Compte, A.M. Cuesta, D. Sanchez-Martin, V. Alonso-Camino, J.L. Vicario, L. Sanz, L. Alvarez-Vallina, Tumor immunotherapy using gene-modified human mesenchymal stem cells loaded into synthetic extracellular matrix scaffolds, Stem Cell (Dayton, Ohio) 27 (2009) 753–760.

[428] K.Y. Lee, N.J. Levell, Turning the tide: a history and review of hyperhidrosis treatment, JRSM Open 5 (2014), 2042533313505511.

[429] D.R. McLachlan, C. Bergeron, J.E. Smith, D. Boomer, S.L. Rifat, Risk for neuropathologically confirmed Alzheimer's disease and residual aluminum in municipal drinking water employing weighted residential histories, Neurology 46 (1996) 401–405.

[430] M.F. Allam, Breast Cancer and Deodorants/Antiperspirants: a Systematic Review, Cent. Eur. J. Public Health 24 (2016) 245–247.

[431] D.M. Pariser, A. Ballard, Topical therapies in hyperhidrosis care, Dermatol. Clin. 32 (2014) 485–490.

[432] A. Lopedota, A. Cutrignelli, V. Laquintana, M. Franco, D. Donelli, L. Ragni, S. Tongiani, N. Denora, β-cyclodextrin in personal care formulations: role on the complexation of malodours causing molecules, Int. J. Cosmet. Sci. 37 (2015) 438–445.

[433] C. Grove, W. Liebenberg, J.L. du Preez, W. Yang, M.M. de Villiers, Improving the aqueous solubility of triclosan by solubilization, complexation, and *in situ* salt formation, J. Cosmet. Sci. 54 (2003) 537–550.